STANDARD OPERATING PROCEDURES

EXTRACTION AND ANALYSIS OF 4-METHYLCYCLOHEXANE METHANOL IN WATER BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) BY SELECTIVE ION MONITORING (SIM)
(MODIFIED EPA/SW-846 Methods 3500C/3510C/8000C/8270D)

CONTENTS

1.0 SCOPE AND APPLICATION

2.0 METHOD SUMMARY

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING AND STORAGE

- 3.1 Sample Storage
- 3.2 Holding Times

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

5.0 EQUIPMENT/APPARATUS

6.0 REAGENTS

7.0 PROCEDURES

- 7.1 Sample Preparation and Extraction
- 7.2 GC/MS Operating Conditions
- 7.3 Decafluorotriphenylphosphine Tune
- 7.4 Initial Calibration
- 7.5 Initial Calibration Verification
- 7.6 Continuing Calibration
- 7.7 Sample Analysis
- 7.8 Identification of Target Analytes
- 7.9 Library Search

8.0 CALCULATIONS

- 8.1 Target Compounds
- 8.2 Tentatively Identified Compounds
- 8.3 Surrogate Spike Recoveries
- 8.4 Matrix Spike Recoveries
- 8.5 Laboratory Control Sample Recoveries

9.0 QUALITY ASSURANCE/ QUALITY CONTROL

- 9.1 GC/MS Tuning and Performance Criteria
- 9.2 GC/MS Initial Calibration
- 9.3 GC/MS Initial Calibration Verification

CONTENTS (cont)

9.4 GC/MS Continuing Calibration
9.5 Internal Standard Area Evaluation
9.6 Method Blank Analysis
9.7 Surrogate Recoveries
9.8 MS/MSD Analysis
9.9 Dilution Analysis
9.10 Manual Integrations
9.11 Laboratory Control Sample
9.12 Demonstration of Capability
9.13 Limit of Detection/Limit of Quantitation Studies
9.14 Nonconformance Memo

10.0 DATA VALIDATION

11.0 HEALTH AND SAFETY

12.0 REFERENCES

13.0 APPENDICES

A – Tables and Figures

EXTRACTION AND ANALYSIS OF 4-METHYLCYCLOHEXANE METHANOL IN WATER BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) BY SELECTIVE ION MONITORING (SIM) (MODIFIED EPA/SW-846 Methods 3500C/3510C/8000C/8270D)

1.0 SCOPE AND APPLICATION

This Standard Operating Procedure (SOP) outlines the preparation and analysis of 4-methylcyclohexane methanol (4-MCHM) in water matrices using a gas chromatograph/mass spectrometer (GC/MS) operated in the selective ion monitoring (SIM) mode. This method is based on modified Environmental Protection Agency (EPA) Methods SW846/3500C/3510C/8000C/8270D. This method can be used to quantitate 4-MCHM that is soluble in methylene chloride and capable of being separated into cis and trans isomers on a fused-silica capillary column. MCHM exists as two diastereomers with similar properties. Table 1 lists the reporting limit (RL) for this compound.

This method may not be changed without the expressed approval of the Analytical Support Leader and the Quality Assurance/Quality Control (QA/QC) Officer. Only those versions issued through the SERAS document control system may be used. Modifications made to the procedure due to interferences in the samples or for any other reason must be documented in the case narrative and on a nonconformance memo.

2.0 METHOD SUMMARY

An aliquot (e.g., 1.0-liter) of a sample is spiked with a surrogate and serially extracted with methylene chloride using a separatory funnel. The extract is subsequently dried and concentrated to a final volume of 10 milliliters (mL). The final extract is spiked with an internal standard and analyzed by GC/MS. 4-MCHM is identified by comparing the measured mass spectra and retention times with those obtained from calibration standards acquired under the same operating conditions used for the samples. Quantitation of this target analyte is calculated based on the internal standard method. Table 2, Appendix A lists the characteristic ions of each target analyte.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING AND STORAGE

3.1 Sample Storage

Water samples should be collected in two 1-liter (L) amber glass containers fitted with Teflon-lined caps. From the time of collection until after analysis, extracts and unused samples must be protected from light and refrigerated at ≤6 degrees Celsius (°C) for the periods specified by SERAS Task Leader (TL) and/or the Work Assignment Manager (WAM) for the project.

Samples and sample extracts must be stored separately from standards in an atmosphere free of all potential contaminants.

3.2 Holding Times

The extraction of water samples will be completed within 7 days of sample collection and analysis completed within 40 days of sample extraction.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Interferences caused by contaminants in solvents, reagents, glassware and other sample processing hardware, may be introduced during extraction and/or analysis. These interferences may interfere with the identification of target compounds and/or tentatively identified compounds (TICs) (i.e., co eluting peaks) or may cause elevated baselines in the total ion chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks on a routine basis. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source.

Phthalate esters are present in many types of products commonly found in the laboratory. Some plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if good laboratory practices (GLP) or consistent quality control (QC) is not practiced.

5.0 EQUIPMENT/APPARATUS

The following equipment/apparatus is typically used during the performance of this SOP. Other standard laboratory equipment may be substituted or added, as appropriate.

- Separatory funnel, 2000 mL with polytetrafluoroethylene (PFTE) stopcock.
- Erlenmeyer flasks, 500 mL
- Funnels
- Pyrex glass wool, baked at 400°C for at least 4 hours prior to use
- Desiccator
- Teflon boiling chips, approximately 10/40 mesh, washed with methylene chloride
- Disposable glass Pasteur pipettes
- TurboVap concentrator, with concentrator cells and racks
- Clean Bath solution, for use in TurboVap II concentrator
- Ohaus digital scale, capable of weighing to 0.001 grams
- S-weights, accompanied by a certificate of analysis

- GC autosampler glass vials with crimp caps, 2 mL
- Agilent Technologies 6890/7890 GC and 5973/5975 mass selective (MS) detector or equivalent, equipped with an autosampler and controlled by the Enviroquant (or equivalent) software
- Zebron, ZB-5 fused silica capillary column, 30 meter (m) x 0.25 millimeter (mm) inner diameter (ID), 0.5 micron (µm) film thickness (or equivalent)
- Syringes, various microliters (µL) volumes, for spiking and preparation of standards
- Micro syringes, 10 µL and larger, 0.006 inch ID needle
- Graduated cylinder, 1L
- Volumetric flasks, Class A, various volumes ranging from 5 to 500 mL
- pH paper, wide range
- Ring stands

6.0 REAGENTS

- Deionized water (DI), organic free reagent water
- Sodium Sulfate (Na_2SO_4), anhydrous granular (12/60 mesh) reagent grade, heated at 400°C for four hours, cooled in a dessicator, stored in a glass bottle
- Methylene Chloride (DCM), pesticide residue analysis grade or equivalent, each lot analyzed prior to use.
- Stock Surrogate Spike Nitrobenzene-d5, neat standard
- Intermediate Surrogate Spike Solution, 10,000 micrograms per milliliter (µg/mL) – Weigh out 0.1 grams (g) of the neat nitrobenzene-d_5 stock surrogate spike and add DCM to a final volume of 10 mL.
- Intermediate Surrogate Spike Solution, 10 µg/mL – Add 10 microliters (µL) of the intermediate 10,000 µg/mL surrogate spike solution to 10 mL of DCM.
- Working Surrogate Spiking Solution, 4.0 µg/mL – Add 20 µL of the intermediate 10,000 µg/mL surrogate spike to a 50-mL Class A volumetric flask. Dilute to volume with DCM.
- Stock Internal Standard 2-Fluorobiphenyl, 96 percent (%) neat standard

- Intermediate Internal Standard Solution, 10,000 µg/mL – Weigh out 0.1 g of the neat 2-fluorobiphenyl stock internal standard and add DCM to a final volume of 10 mL.

- Intermediate Internal Standard Solution, 2000 µg/mL – Add 200 µL of the 10,000 µg/mL intermediate internal standard solution to 1.0 mL of DCM.

- Working Internal Standard Solution, 25 µg/mL – Add 125 µL of the 2000 µg/mL intermediate internal standard solution to 10 mL of DCM. Twenty µL of the internal standard (IS) solution is added to each 1 mL of the sample extract before analysis, resulting in a 500 micrograms per liter (µg/L) injection.

- Primary Stock 4-MCHM Calibration Standard, neat

- Primary Intermediate 4-MCHM Calibration Standard, 10,000 µg/mL – Weigh out 0.1 g of the neat primary stock 4-MCHM and add DCM to a final volume of 10 mL.

- Primary Intermediate 4-MCHM Calibration Standard, 10 µg/mL – Add 10 µL of the 10,000 µg/mL 4-MCHM calibration standard to 10 mL of DCM.

- Primary 4-MCHM Calibration Working Standards, 50, 100, 200, 500 and 1000 µg/L – Prepare as follows in DCM:

Concentration (µg/L)	µL of 10 µg/mL Intermediate 4-MCHM Std.	µL of 10 µg/mL Intermediate Nitrobenzene-d5	µL of 25 µg/mL Internal Std (added to 1 mL of standard)	Final Volume (µL)
50	50	50	20	10000
100	100	100	20	10000
200	200	200	20	10000
500	500	500	20	10000
1000	1000	1000	20	10000

Stock Decafluorotriphenylphosphine (DFTPP) Mix, 1000 µg/mL, consisting of pentachlorophenol, DFTPP, benzidine and 4,4'-DDT each at 1000 µg/mL

- DFTPP Tune Solution, 50 µg/mL – Dilute 500 µL of the Stock 1000 µg/mL DFTPP to 10 mL of 65:35 v/v DCM/hexane. Add 200 µL of the stock 2,000 µg/mL internal standard mix. The amount of DFTPP in a 1-µL injection is 50 nanogram (ng).

- Secondary 4-MCHM Stock Standard, neat

- Secondary 4-MCHM Intermediate Standard, 10,000 µg/mL – Weigh out 0.1 grams (g) of the neat secondary 4-MCHM stock standard and add DCM to a final volume of 10 mL.

- Secondary 4-MCHM Working Standard, 10 µg/mL – Add 10 µL of the secondary 4-MCHM 10,000

µg/mL intermediate standard to 10 mL of DCM.

- Secondary 4-MCHM Working Spiking Solution, 4.0 µg/mL – Add 4 µL of the Secondary 10 µg/mL 4-MCHM intermediate standard to 10 mL of DCM.

- Initial Calibration Verification Standard, 200 µg/L – Add 200 µL of the Secondary 10 µg/mL 4-MCHM intermediate standard to 10 mL of DCM.

- Nitrogen, high purity, for evaporation

- Helium, ultra high purity grade for GC/MS system

NOTE: All of the above mentioned standard solutions must be stored at <6°C in a dedicated refrigerator/freezer in tightly capped amber vials with Teflon liners. Commercially prepared standard solutions that are received in sealed ampoules may be stored in the shelf section of the standards refrigerator.

NOTE: Premixed certified standards will be stored according to the manufacturer's documented storage requirements. These standards may be kept in storage up to the manufacturer's stated expiration date. Once the standard vials are opened, the standards will be stored with minimal headspace in the freezer (-10°C) for a period not to exceed six months or the manufacturer's expiration date, whichever is less.

NOTE: The IS mixture should be stored in the refrigerator at 4°C.

NOTE: All calibration standards, surrogates, internal standards, and spiking solutions will be prepared and documented in accordance with SERAS SOP #1012, *Preparation of Standard Solutions and Reagents.*

7.0 PROCEDURES

7.1 Sample Preparation and Extraction

1. Mark the water meniscus on the sample bottle (using a grease pencil or a marker).

2. Rinse a 2-L separatory funnel and a receiving flask two to three times with methylene chloride.

3. Pour the entire contents of a well-mixed 1-L sample into a separatory funnel.

4. Transfer 1-L of DI water to a 2-L separatory funnel to be used as a method blank. A method blank must be prepared with every batch not to exceed 20 samples.

5. Transfer 1-L of DI water to a 2-L separatory funnel to be used as a laboratory control sample (LCS). A LCS must be prepared for every 20 samples.

6. Transfer two 1-L aliquots of the sample chosen for spiking to two separate 2-L separatory funnels. A matrix spike/matrix spike duplicate (MS/MSD) must be prepared for every 20 samples or per project if sufficient volume has been submitted.

   NOTE: The sample to be used for this purpose may be specified on the Chain of Custody (COC) Record.

7. Check and record the sample pH using wide-range pH paper. For 4-MCHM, the extraction will be done at a neutral pH.

8. Add 500 µL of the working surrogate spiking solution to the method blank, LCS, MS/MSD and all environmental samples.

9. Add 500 µL of the Secondary 4.0 µg/mL 4-MCHM Working Spiking Solution to the LCS and MS/MSD.

10. Rinse the empty sample bottle with 60 mL of methylene chloride. Transfer the rinsate to the separatory funnel and stopper the flask.

11. Gently, invert the separatory funnel and vent to release any pressure. Once vented, shake the separatory funnel for approximately three minutes with proper venting techniques to avoid loss of the extract.

12. Place the separatory funnel back into the ring and allow the layers to separate for 5 to 10 minutes. If the separation is not achieved after 10 minutes, use other mechanical techniques, such as centrifuging, glass rod stirring, or smaller separatory funnels to separate the layers.

13. After separation is completed, filter the organic layer (bottom layer) through a funnel containing glass wool and anhydrous sodium sulfate into a 500-mL Erlenmeyer receiving flask.

14. Repeat steps 12 through 14 two more times. The sample must be serially extracted a total of three times.

15. Fill the TurboVap water bath with approximately one gallon of DI water mixed with 10 to 15 drops of Clean Bath solution. Set the water temperature at 45°C.

16. In a fume hood, transfer as much of the combined extracts into 200-mL concentrator tubes. Place the tubes into the TurboVap and begin concentrating by blowing a gentle stream of nitrogen into the tubes so that no solvent splashes out. As the solvent level is reduced, add the remaining extract. Once all of the extract has been added to the concentrator tube and the solvent level is below the 200-mL mark, the flow of nitrogen can be increased to speed up the concentration. Periodically rinse the sides of the tube with methylene chloride.

17. Concentrate the extract until the solvent only remains in the stem of the concentrator tube. This is visible by looking straight down into the cell to see solvent only in the inner circle. At this point, the extract volume is approaching 10 mL and should be monitored carefully. Do not let the extract evaporate to dryness. When removing the cell to add or transfer contents, ensure water bath droplets do not fall into the other extracts.

18. Transfer the extract into a 10 mL vial and adjust the final volume to 10 mL with DCM. Transfer 1 mL of extract into 2-mL GC autosampler vial. The extract is ready for analysis.

19. Refill each sample bottle with water up to the mark. Measure the volume of water using a Class A graduated cylinder. Record the volume of each sample in the extraction log.

7.2 GC/MS Operating Conditions

The following GC/MS operating conditions are recommended for the Agilent Technologies 6890/7890 GC and 5973/5975 MS detector system (refer to Table 4, Appendix A for complete listing):

Column	Zebron ZB-5 (5% phenyl - 95% dimethyl-polysiloxane) 30 meter x 0.25 mm ID, 0.50 µm film
Flow rate	1.5 mL/min., EPC enabled
Injection Temperature	270°C
Transfer Line/Aux Temp.	325 °C
Source Temperature	280 °C
Quad Temperature	180 °C
GC Oven Temp. Program	50°C, hold 1.0 minute, ramp at 17.5 °C/min to 200 °C, hold 0.5 min, ramp at 35 °C/min to 315 °C, hold for 2 minutes[1]
Pulsed Split Injection	Split ratio = 12:1, split flow = 18 mL/min, pulse = 22 psi for 0.5 min Gas Saver 35 mL/min. at 0.75 min
Injection Volume	2µL Use 4mm ID, single gooseneck liner packed with silanized glass wool

SIM Parameters Group 1
Resolution: Low
Plot 1 Ion: 54

Ions/Dwell in Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(54.00 50)	(55.00 50)	(82.00 50)
	(95.00 50)	(97.00 50)	(128.00 50)
	(171.00 50)	(172.00 50)	

7.3 Decafluorotriphenylphosphine Tune

The MS must be tuned with PFTBA so that a 50 ng injection of DFTPP produces spectra that will meet the ion abundance criteria listed in Table 3, Appendix A. The tune is acquired by averaging the apex and ± one scan. Background subtraction should be done using a single scan no more than 20 scans prior to the elution of the DFTPP and should be used to eliminate column bleed or instrument background noise. The DFTPP tune criteria must be met every 12 hours during sample analysis. If the software does not indicate what scan was subtracted, the analyst will document the scan number directly on the tune report. The tuning standard should also contain 50 nanograms per microliter (ng/µL) of 4,4'-DDT, pentachlorophenol, and benzidine to verify injection port inertness and GC column performance. Degradation of DDT to DDE and DDD is measured and should not exceed 20%. Benzidine and Pentachlorophenol should be present at their normal responses, and should not exceed a tailing factor of 2 given by the following equation:

$$\text{Tailing Factor} = BC/AB$$

Where:
BC = the right side half of the peak width, measured from 10% up the baseline of the peak
AB = the left side half of the peak width, measured from 10% up the baseline of the peak

Figure 1, Appendix B illustrates how to determine the tailing factor.

Note: The Enviroquant software is capable of automatically calculating the degradation and tailing factors and should be used whenever possible.

7.4 Initial Calibration

1. Add 20 µL of the internal standard mix to each 1 mL aliquot of the five calibration standards. Do not add the internal standard if using commercially prepared calibration standards that already contain the internal standards.

2. After DFTPP passes the criteria, set up the run using the six calibration standards.
NOTE: A minimum of 5 standards must be used to generate the initial calibration curve.

3. Calculate and tabulate the relative response factor (RRF) against the concentration for each compound, including the surrogates, by using the equation below. The primary ion from the specific internal standard must be used for quantitation. The average RRF and percent relative standard deviation (%RSD) must also be calculated and tabulated.

4. The %RSD must be less than 20% for all compounds or a minimum correlation coefficient of 0.99 using linear regression. Since the method of linear regression analysis has the potential for a significant bias to the lower portion of a calibration curve, the recalculated concentration of the low calibration point should be within ± 30% of the standard's true concentration.

$$RRF = \frac{A_x \times C_{IS}}{A_{IS} \times C_x}$$

where:

A_X	=	Area of the characteristic ion of each target analyte
A_{IS}	=	Area of the characteristic ion of each internal standard assigned to target analytes
C_{IS}	=	Concentration of each internal standard, nanograms per microliter (ng/µL)
C_X	=	Concentration of each target analyte (ng/µL)

5. Use the following equations to calculate and tabulate average RRF and %RSD for 4-MCHM.

$$\overline{RRF} = \frac{\sum_{i=1}^{n} RF_i}{n}$$

where:

$\overline{RRF}$	= relative response factor for each initial calibration level
N	= total number of initial calibration levels

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (RRF_i - RRF_{avg})^2}{n-1}}$$

$$\%RSD = \frac{SD}{RRF_{avg}} x\ 100$$

where:

RRFi	=	individual RRF
RRF_{avg}	=	average RRF
n	=	number of calibration standards

7.5 Initial Calibration Verification

The initial calibration curve must be verified immediately after performing the ICAL using a 2nd source standard. The suggested initial acceptance limits for the ICV analysis are 70-130%.

7.6 Continuing Calibration

1. A check of the initial calibration curve must be performed every 12 hours after an acceptable DFTPP analysis. Sample analysis may begin only after a successful DFTPP tune and a continuing calibration check have been acquired.

2. Inject 2 µL of a 200 µg/L calibration standard that contains the target analyte, surrogate and internal standard. The internal standard concentration is 500 µg/L.

3. Calculate and tabulate the continuing calibration RRF for each compound.

4. Calculate the percent difference (%D) for the continuing calibration RRF compared to the average RRF from the initial calibration curve.

$$\%D = \frac{\left|(RRF_{Daily} - RRF_{Avg})\right|}{RRF_{Avg}} \times 100$$

Where:
RRF_{Daily} = RF of continuing calibration

The criteria for the continuing RRF and %D are found in Section 9.4.

5. The extracted ion current profile (EICP) area for each internal standard in the continuing calibration must be compared to the internal standard area in the mid-point standard of the current initial calibration. The criterion for comparison is found in section 9.4.

6. If the percent difference or percent drift for a compound is less than or equal to 20%, then the initial calibration for that compound is assumed to be valid. If the criterion is not met (i.e., greater than 20% difference or drift), then corrective action must be taken prior to the analysis of samples.

7.7 Sample Analysis

Prior to the analysis of calibration standards, blanks, and/or samples, it is necessary to verify that the GC/MS:

- Met the DFTPP ion abundance criteria listed in Table 3, Appendix A and in Section 9.1. The DFTPP tune criteria must be demonstrated every 12 hours by analyzing 50 ng of DFTPP.

- Successfully passed an initial five-point calibration and/or continuing calibration check. The continuing calibration check must be demonstrated every 12 hours during sample analysis by analyzing a 200 µg/L MCHM standard.

The method blanks, LCS, MS/MSD and samples must be analyzed with the same instrument conditions used for the calibration standards.

1. Add 20 µL of the internal standard mix into the method blank, LCS, MS/MSD, and all sample extracts.

2. Inject 2 µL of the extract for each method blank, LCS, MS/MSD or sample.

3. If the response exceeds that of the highest calibration standard, the extract must be diluted so that the analyte response falls within the linear range established in the initial calibration. Ideally, the concentration of the analyte should fall midrange of the curve after dilution.

4. After a dilution is prepared, the internal standard mix is added accordingly, to maintain the required concentration of 500 µg/L in the diluted extract.

7.8 Identification of Target Analytes

The target analytes are identified by comparison of the sample mass spectra with the mass spectra of a calibration standard. Two criteria must be satisfied to verify the identifications:

- Elution of the sample component at the GC relative retention time (RRT) as the standard component

- Correspondence of the sample component and standard component mass spectra

1. For establishing correspondence of the RRT, the sample component RRT must compare within ±0.06 RRT units of the RRT of the standard component. For reference, the standard must be run on the same shift as the sample. If co-elution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using extracted ion current profiles for ions unique to the component of interest.

2. For comparison of standard and sample components, reference mass spectra must be obtained from the 200 µg/L calibration standard. The standard mass spectra may be

obtained from the run used to obtain the reference RRTs. In the case of co-elution of standard components, the reference mass spectra from the National Institute of Standard and Technology (NIST) Mass Spectral Library should be used or the analyst can use professional judgment to establish the presence of target analytes. When technical judgment is used, the analyst must document the circumstances and their observations in the case narrative.

3. The requirements for qualitative verification of mass spectra are as follows:

   a. The three ions of greatest relative intensity must be present or any ions over 30% relative intensity if less than three such ions exist.

   b. The relative intensities of ions specified in (a) must agree within ±30% between the standard and sample spectra. For example, if an ion with an abundance of 50% in the standard spectra, the corresponding sample ion abundance must be between 20-80%.

   c. Ions greater than 10% present in the *sample* spectrum but not in the *standard* spectrum must be considered and accounted for by the analyst making the comparison. Report the actual value of all target analytes below the quantitation limit with a flag of "J", e.g., "3 J".

4. If the compound cannot be verified by all of the criteria in Step 3 but is identified by the technical judgment of the mass spectral interpretation specialist, the analyst shall report that identification and proceed with the calculation described in Section 8.0. The analyst should report in the case narrative the reasons why the compound is identified.

8.0 CALCULATIONS

8.1 Target Compounds

4-MCHM must be quantitated by the internal standard method. The extracted ion current profile (EICP) area of the primary ion of 4-MCHM listed in Table 2, Appendix A is used for quantitation.

Whether the sample is analyzed after initial calibration or daily continuing calibration, the average relative response factor (RRF_{avg}) is used to calculate the concentrations of identified analytes based on the following equation:

$$Concentration\ (\mu g/L) = \frac{(A_X)(I_S)(V_t)(DF)}{(A_{is})(RRF)_{avg}(V)}$$

where:

A_X = Area of the characteristic ion of each target analyte
I_S = Amount of each internal standard injected (µg/mL)

V_T	=	Volume of the concentrated extract (mL)
DF	=	Dilution factor
A_{IS}	=	Area of the characteristic ion of each internal standard
RRF_{avg}	=	Average relative response factor
V	=	Volume of water sample extracted (L)

The following EPA-defined flags will be used in the lab to qualify data:

U: This flag indicates that the compound was analyzed for but not detected

J: This flag indicates an estimated value above the limit of detection but under the sample RL

B: This flag is used when the analyte is found in the associated method blank as well as in the sample

E: This flag identifies compounds whose concentrations exceed the upper calibration range of the instrument

The target concentration is reported to three significant figures. For any concentrations reported from a diluted run, be sure to report the corresponding RL. For example, if the sample is run at a 10x dilution to bring the concentration within linear range, the RL must be reported at 5.0 µg/L instead of 0.5 µg/L. The RL is based on the lowest standard from the calibration curve multiplied by any dilution factor.

8.2 Surrogate Spike Recovery

Calculate surrogate standard recovery on all samples, blanks, and spikes by the following equation:

$$\textit{Percent Recovery} = \left(\frac{Q_D}{Q_A}\right) 100x$$

where:

Q_D	=	Quantity determined by analysis
Q_A	=	Quantity added to sample

8.3 Matrix Spike Recoveries

Accuracy is calculated from the recovery of the MS/MSDs. Precision is calculated from the relative percent difference (RPD) of the recoveries measured for the MS/MSD pair. Matrix spike recoveries and RPD will be calculated by the following equations:

$$\text{Matrix Spike Recovery (\%R)} = \frac{SR-MS}{SA} 100x$$

where:

MS	=	Concentration of target analyte in spike sample (spiked)
SR	=	Concentration of target analyte in sample (unspiked)
SA	=	Concentration of spike added

and

$$RPD = \frac{|MSD-MS|}{(MS+MSD)/2} 100x$$

where:

RPD	=	Relative percent difference
MS	=	Matrix spike concentration
MSD	=	Matrix spike duplicate concentration

Note: RPD is always expressed as a positive value.

8.4 Laboratory Control Sample Recovery

The recoveries of 4-MCHM in the LCS solution will be calculated using the following equation:

$$\text{Laboratory Control Sample Recovery (\%R)} = \left[\frac{LCS - BLK}{SA}\right] x\,100$$

where:

LCS	=	Concentration of target analyte in LCS
BLK	=	Concentration of target analyte in blank
SA	=	Concentration of spike added

9.0 QUALITY ASSURANCE/ QUALITY CONTROL

9.1 GC/MS Tuning and Performance Criteria

The GC/MS tune must be verified using DFTPP. The ion abundance criteria listed in Table 3, Appendix A must be met prior to any standard, blank or sample analysis. In addition, the criteria must be achieved during every 12-hour period during which standards, blanks, and samples are analyzed. The 12-hour time period for GC/MS tuning begins at the time of DFTPP injection that the laboratory submits as documentation of a compliant tune. The degradation of DDT to DDE

and DDD should not exceed 20%. The tailing factor for benzidine and pentachlorophenol must not exceed a tailing factor of 2.

Figure 1, Appendix B shows an example calculation of the tailing factor. **Figure 2, Appendix B** illustrates the tailing factor for pentachlorophenol and benzidine, and the % breakdown of DDT calculated using the HP Enviroquant software. This feature is found in the *ChromEval* options of *Qedit* in the data analysis mode.

9.2 GC/MS Initial Calibration

4-MCHM must meet the minimum acceptable response factor (RF) 0f 0.05. The %RSD should be less than or equal to 20% or the minimum correlation coefficient (r) criterion of 0.99 for 4-MCHM using linear regression. If the compound criteria is >20% but less than 40%, the compound will be qualified estimated and documented in the case narrative of the analytical data package. The analyst is responsible for determining if maintenance should be conducted prior to the analysis of any additional samples. If 4-MCHM included with the initial calibration exceeds the 20% RSD or the minimum 0.99 correlation coefficient, then the chromatographic system is considered too reactive for analysis to begin and corrective action must be taken. If linear regression is used, then the low point concentration is recalculated using the curve and should be ±30% of its true concentration.

NOTE: All initial calibration standards must be analyzed prior to the analysis of any method blanks, QC samples or environmental samples.

9.3 GC/MS Initial Calibration Verification

The initial calibration curve must be verified immediately after performing the ICAL using a 2nd source standard (ICV). The suggested acceptance limits for the ICV are 70-130%.

9.4 GC/MS Continuing Calibration

After 12 hours of sample acquisition have passed, the GC/MS tune must be re-evaluated using DFTPP, and the initial calibration curve verified by analyzing a mid-level calibration standard.

1. The DFTPP tune must pass the criteria in Table 3, Appendix A.

2. The 200 µg/L calibration standard must be used for the continuing calibration.

3. The %D should be less than or equal to 20% for 4-MCHM and must meet the RRF limit of 0.05. The EICP area for the internal standard in the continuing calibration must be between 50% and 200% of the respective internal standard EICP area in the mid-point standard of the current initial calibration. If this criterion is not met, re-analysis is required.

4. A maximum of two continuing calibrations may be run to meet the requirements in item 3 above. If two continuing calibrations fail to meet acceptable criteria, corrective action must be taken prior to a new calibration curve being run.

   If the instrument is set up on an overnight run with two continuing calibrations back to back and the first continuing calibration passes but the second one fails, then the data is not usable and corrective action must be taken. It is not acceptable to use the first continuing calibration if the second continuing calibration is out of acceptance criteria.

5. If any of the requirements listed in Step 3 are not met, the Analytical Support Leader should be notified so appropriate corrective actions can be taken.

9.5 Internal Standard Area Evaluation

1. The amount of each internal standard in a 2-µL injection of sample extract must be 500 µg/L.

2. The EICP of the internal standards must be monitored and evaluated for each sample, method blank, LCS, MS and MSD.

3. If samples, method blanks, LCS or MS/MSDs are analyzed immediately following an initial calibration but before another DFTPP tune and a continuing calibration, the evaluation will be conducted on the basis of the internal standard areas of the 200 µg/L initial calibration standard.

4. The EICP area for each internal standard in all samples, method blanks, and matrix spike/matrix spike duplicates must be between 50% and 200% of the respective internal standard EICP area in the appropriate calibration standard. In addition, the retention time of each internal standard must be within ±0.50 minutes (30 seconds) of its retention time in the continuing calibration standard.

5. If one or more internal standard EICP areas do not meet criteria, the GC/MS system must be inspected for malfunctions and corrections made as appropriate. When corrections are made, re-analysis of all affected samples is required.

6. If after re-analysis, the EICP areas for all internal standards meet criteria (between 50% and 200%), then the problem with the first analysis is considered to have been within the control of the laboratory. Therefore, only data from the analysis with EICPs within the limits are required to be submitted. If re-analysis confirms matrix effects, submit both sets of data but report the initial run.

9.6 Method Blank Analysis

A method blank is a one liter aliquot of deionized water that is carried through the entire analytical

procedure. The purpose of a method blank is to determine the level of contaminations associated with preparation and analysis of samples.

1. One method blank must be prepared for each batch of 20 samples.

2. A method blank should contain less than the RL of 4-MCHM. If a method blank exceeds the contamination limits as described above, the analytical system is considered unacceptable. The sources of contamination must be investigated so that appropriate corrective actions can be taken and documented before proceeding with any further sample analysis. All samples processed with a contaminated method blank must be re-extracted and reanalyzed.

9.7 Surrogate Recovery

The purpose of using surrogates is to evaluate the accuracy and precision associated with the preparation and analysis of samples. The recovery of the surrogate is calculated for all samples, method blanks, LCS and MS/MSD.

1 The surrogates are added to all samples, method blanks, LCS and MS/MSD prior to extraction.

2 The surrogate recoveries are calculated using the equation in Section 8.2.

3 Surrogate recovery limits must be established when enough data points are available and updated on a quarterly basis by the laboratory. Until that time, a limit 0f 50-150% will be used.

4 If the surrogate is outside QC limits, the following actions must be taken:

a. Ensure that there are no errors in calculations, surrogate solutions, and internal standards. Check that the integration of the quantitation ions of the internal standard and surrogate has been performed properly.

b. If no problems are found in the above step, then re-analyze the sample(s).

c. Do not reanalyze diluted samples with a dilution ratio greater than 10.

d. If the sample associated with the MS/MSD does not meet QC limits, it should be reanalyzed only if the MS/MSD recoveries are within the limits. If the sample and the associated MS/MSD show the same pattern (i.e, outside the limits), the sample does not require reanalysis. Document in the case narrative.

NOTE: Do not reanalyze the MS/MSD, even if their surrogate recoveries fall outside the QC limits.

5 If upon reanalysis of the sample, the surrogate recovery falls within the QC limits, then the problem was within the laboratory's control. Submit only the data from the analysis with the surrogate recovery within the QC limits. This will be considered the initial analysis and reported in the data package. If the reanalysis is outside the analysis holding time, both sets of data will be submitted in the data package.

6 If upon reanalysis of the sample the surrogate recovery still falls outside the QC limits, the sample must be re-extracted and reanalyzed as instructed by the Analytical Support Leader. If the reextraction and reanalysis of the sample solves the problem, submit only the data from the analysis with surrogate recovery within the QC limits. This shall be considered the initial analysis and shall be reported in the data package. If the re-extraction is outside the holding time, provide the data from both analyses.

a. If surrogate recovery in a method blank does not meet QC limits after reanalysis, all samples associated with that blank must be re-extracted with the blank. The blank is intended to detect contamination in samples processed at the same time.

b. If upon reanalysis of the sample associated with MS/MSD, the surrogate recoveries still fall outside the QC limits, the sample must be reextracted

7 If upon reextraction and reanalysis of the sample, the surrogate recovery falls within the QC limits, submit data only from this analysis if the holding time criteria have been met. This shall be considered the initial analysis and will be reported in the data package. If the reextraction is outside the holding time, submit data from both analyses.

8 If upon reextraction and reanalysis of the sample, the surrogate recovery falls outside the QC limits, submit both sets of data. Distinguish between the initial analysis and the reanalysis in the data package.

9.8 MS/MSD Analysis

The purpose of the MS/MSD is to evaluate the accuracy and precision of the extraction and analysis, including possible sample matrix effects.

1. A MS/MSD must be analyzed every 20 samples or per project. The MS/MSD must be associated with a method blank that meets the criteria in section 9.6, a calibration in sections 9.2 and 9.4 and a tune in section 9.1. The MS/MSDs should be run on the same 12-hour shift as the spiked sample.

2. The MS/MSD recovery limit is 50-150% and RPD limits are ±20% until enough data points are available to create control chart limits. The control charts will be updated on a quarterly basis by the laboratory. The Analytical Support Leader or their designee will verify the accuracy of the control chart data.

Note: If the laboratory fails to meet the recovery QC limits on a routine basis, the Analytical Support Leader must investigate the cause and take corrective action.

MS/MSD recovery outliers must be documented on a nonconformance memo and stated in the associated case narrative. A matrix effect is indicated if the LCS data are within limits but the MS/MSD data are not. A similar pattern should be observed for both the MS and MSD.

The MS/MSD must be prepared at the same dilution as the least diluted analysis from which sample results will be reported.

9.9 Dilution Analysis

If the concentration of 4-MCHM in a sample extract exceeds the initial calibration range, the sample extract must be diluted and reanalyzed as described in Section 7.7.

1. Use the results from the initial analysis to estimate the approximate dilution factor needed to bring the highest concentration within the linear calibration range.

2. The dilution factor chosen should bring the highest target analyte within the upper half of the calibration range.

3. Submit the data from the original sample and the dilution in which analytes fall within the calibration range. If the screening procedure determines that the extract cannot be analyzed undiluted, submit the data from the first dilution and a subsequent dilution in which analytes fall within the calibration range. NOTE: Except in extreme cases, all extracts should be run undiluted to achieve the lowest detection limit.

9.10 Manual Integrations

Manual integration of all target analytes, surrogates, and internal standards for standards, samples and QC samples will be submitted for review. The manual integration results will be flagged with a "M" and will be initialed and dated by the analyst indicating that the integration was performed properly. A reason for the manual integration should be documented if not readily obvious to the data reviewer. Documentation of the manual integration (before and after total ion chromatograms) of quantitation ion peaks must be included in the data package. Refer to SERAS SOP #1001, *Chromatographic Peak Integration Procedures* for more details.

9.11 Laboratory Control Sample

A LCS must be analyzed every 20 samples or per batch. The LCS should be prepared at 200 µg/L (or another concentration near the mid-point of the calibration) from a second source working standard. The LCS must be associated with a method blank that meets the criteria in section 9.6, a

calibration in sections 9.2 through 9.4 and a tune in section 9.1.

The acceptance criteria will be 50 to 150% until the sufficient data points are available to prepare a control chart. At that point, control and warning limits will be calculated every 10 to 20 points and updated at least quarterly. All LCS recoveries should be documented in the associated case narrative.

If the lab fails to meet the QC recovery limits on a routine basis, the Analytical Support Leader must investigate the cause and take corrective action.

9.12 Demonstration of Capability

Prior to running a method or any time there is a change in instrument type, personnel or test method or any time a method or analyst has not performed a method in a twelve-month period, an analyst must demonstrate acceptable performance for that method. This is known as the initial demonstration of capability (DOC). Four aliquots of a QC standard (second source) must be prepared and analyzed according to the method over one or a period of days. If the method does not specify a concentration to be used for the DOC, then the concentration must be prepared at 1-4 times the RL. Using Excel, the analyst must provide a table containing the following: Results of the 4 replicates, Mean Concentration, Mean Recovery, Standard Deviation and %RSD with the LCS acceptance limits.

On-going demonstration of capability on an annual basis may be satisfied by the analyst successfully passing a performance testing (PT) sample or a minimum of 4 consecutive LCS samples with acceptable precision and accuracy levels or another initial DOC. Results of tabulated QC samples (i.e., control chart data) that are analyst-specific accompanied by precision and accuracy criteria agreed upon by the Analytical Support Leader and the QA/QC Officer may also be used.

9.13 Limit of Detection/Limit of Quantitation Studies

The LOD must be determined for each target analyte on every instrument that will be used for the analysis and reporting of samples. The LOD must be determined each time there is a change in the method that affects how the test is performed or when there is a change in instrumentation. The LOD must be verified annually for each matrix, method and analyte. The LOD will be run using a minimum of seven replicates of a sample prepared from the calibration source at 4 times the LOD for multiple analyte tests. Each of these 7 aliquots must be subjected to the entire analytical procedure. Calculate the mean, mean recovery, variance and standard deviation of the replicate measurements. The LOD is calculated by multiplying the standard deviation times the Students t-Value of 3.143. If more than 7 replicates are used, the Students t-Value must be adjusted accordingly.

The LOQ must be confirmed by the successful analysis of a secondary source QC sample containing each target analyte in each matrix at 1-2 times the claimed LOQ (typically the low

standard). The recoveries must fall within the recoveries established for the LCS. Alternately, a control chart with recoveries established from previously analyzed LOQ samples may be used. The LOQ must be subjected to the entire analytical procedure. The LOQ must be verified annually for each matrix, method and analyte. The LOD/LOQ must be determined at the same time.

9.14 Nonconformance Memo

A nonconformance memo will be generated any time an employee notices a deficiency suspected of being a nonconformance. This nonconformance memo will be forwarded to the QA/QC Officer for verification of corrective action.

10.0 DATA VALIDATION

Data will be assessed in accordance with the guidelines set forth in the most current version of SERAS SOP #1016, *Data Validation Procedures for Routine Semivolatile Organic Analysis*. However, data are considered satisfactory for submission when all the following requirements are met.

1. All samples must be analyzed under an acceptable tune, initial calibration, and continuing calibration check at the required frequency.

2. The QC requirements described in Section 9.0 should be met at all times. Any deviation or anomalous conditions should be discussed with the Analytical Support Leader and documented on a nonconformance memo.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, refer to EPA, Occupational Safety and Health Administration (OSHA) and corporate health and safety practices. More specifically, refer to SERAS SOP #3013, *Chemical Hygiene Plan* and SERAS SOP #1501, *Hazardous Waste Management*.

12.0 REFERENCES

U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. 2007. *Test Methods for Evaluating Solid Waste*. SW-846. 3rd ed. Method 3500C, Revision 3.

U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. 1996. *Test Methods for Evaluating Solid Waste*. SW-846. 3rd ed. Method 3510C, Revision 3.

U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. 2007. *Test Methods for Evaluating Solid Waste*. SW-846. 3rd ed. Method 8270D, Revision 4.

U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. 2003. *Test Methods for Evaluating Solid Waste*. SW-846. 3rd ed. Method 8000C, Revision 3.

U.S. EPA. 1984. Federal Register, 40 Code of Federal Regulations (CFR) Part 136, Appendix B, *Definition and Procedure of the Determination of the Method Detection Limit - Revision 1.11*, October 26, 1984.

13.0 APPENDICES

A –Tables

APPENDIX A
Tables
SOP #1857
January 2014

TABLE 1. Target Compound List and Reporting Limit

ANALYTE	RL[1] (µg/L)
4-Methylcyclohexane methanol	0.500

TABLE 2. Characteristic Ions for Target Compounds and Surrogates

Analyte	Primary Ion	Secondary Ion(s)
2-Fluorobiphenyl (ISTD)[1]	172	171
Nitrobenzene-d5 (SS)	82	54, 128
4-Methylcyclohexane methanol	55	97, 95

TABLE 3. Ion Abundance Criteria for Tune (DFTPP) [1]

Mass	Ion Abundance Criteria
51	10.0 - 80.0 percent of mass 198
68	Less than 2.0 percent of mass 69
70	Less than 2.0 percent of mass 69
127	10 – 80 percent of mass 198
197	Less than 2.0 percent of mass 198
198	Base peak, 100 percent relative abundance (see note)
199	5.0 - 9.0 percent of mass 198
275	10.0 – 60.0 percent of mass 198
365	Greater than 1.0 percent of mass 198
441	Present but < 24% of mass 442
442	Base Peak, or > 50% of mass 198
443	15.0 - 24.0 percent of mass 442

NOTE: All ion abundances MUST be normalized to m/z 198, the nominal base peak, even though the ion abundances of m/z 442 may be up to 110 percent that of m/z 198.

[1] Criteria taken from U.S. EPA/SW846, Method 8270D, Revision 4.0, February 2007

TABLE 4. Recommended GC/MS Instrument Parameters

Sample Inlet:	GC
Injection Source:	GC ALS
Mass Spectrometer:	Enabled
Oven	
Equilibration Time	0.5 min
Oven Program	On
	50 °C for 1 min
	then 17.5 °C/min to 200 °C for 0.5 min
	then 35 °C/min to 315 °C for 2 min
Run Time	15.357 min
Front Injector	
Syringe Size	10 µL
Injection Volume	2 µL
Solvent A Washes (PreInj)	2
Solvent A Washes (PostInj)	5
Solvent A Volume	8 µL
Solvent B Washes (PreInj)	2
Solvent B Washes (PostInj)	5
Solvent B Volume	8 µL
Sample Washes	2
Sample Wash Volume	5 µL
Sample Pumps	2
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min
Sample Wash Draw Speed	300 µL/min
Sample Wash Dispense Speed	6000 µL/min
Injection Dispense Speed	6000 µL/min
Viscosity Delay	0 sec
Sample Depth	Disabled
Front SS Inlet He	
Mode	Pulsed Split
Heater	On 270 °C
Pressure	On 13.329 psi

TABLE 4. Recommended GC/MS Instrument Parameters (cont.)

Total Flow	On	22.5 mL/min
Septum Purge Flow	On	3 mL/min
Gas Saver	On	35 mL/min After 0.75 min
Split Ratio	12:1	
Split Flow	18 mL/min	
Injection Pulse Pressure	20 psi Until 0.5 min	

Thermal Aux 2 {MSD Transfer Line}	
Heater	On
Temperature Program	On
	325 °C for 0 min
Run Time	15.357 min

Column #1	
ZB-5	Serial # 140533
	30 m x 250 µm x 0.5 µm
	In: Front SS Inlet He
	Out: Vacuum
(Initial)	50°C
Pressure	13.239 psi
Flow	1.5 mL/min
Average Velocity	43.91 cm/sec
Holdup Time	1.1387 min
Flow Program	Off
Run Time	15.357 min

MS ACQUISITION PARAMETERS

General Information
--- ---

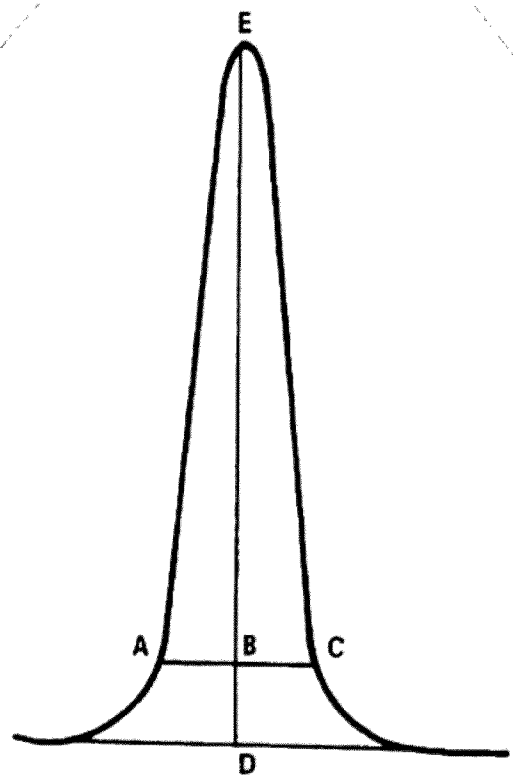
Tune File:	Xtune.U
Acquisition Mode:	SIM

TABLE 4. Recommended GC/MS Instrument Parameters (cont.)

MS Information
-- ----------

Solvent Delay:	2.20 min		
EMV Mode:	Relative		
Relative Voltage:	47		
Resulting EM Voltage:	1635		
[SIM Parameters]			
GROUP 1			
Resolution: Low			
Plot 1 Ion: 54			
Ions/Dwell in Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(54.00 50)	(55.00 50)	(82.00 50)
	(95.00 50)	(97.00 50)	(128.00 50)
	(171.00 50)	(172.00 50)	
[MSZones]			
MS Source:	280 C maximum 300ºC		
MS Quad:	180 C maximum 200ºC		

FIGURE 1. Tailing Factor Calculation

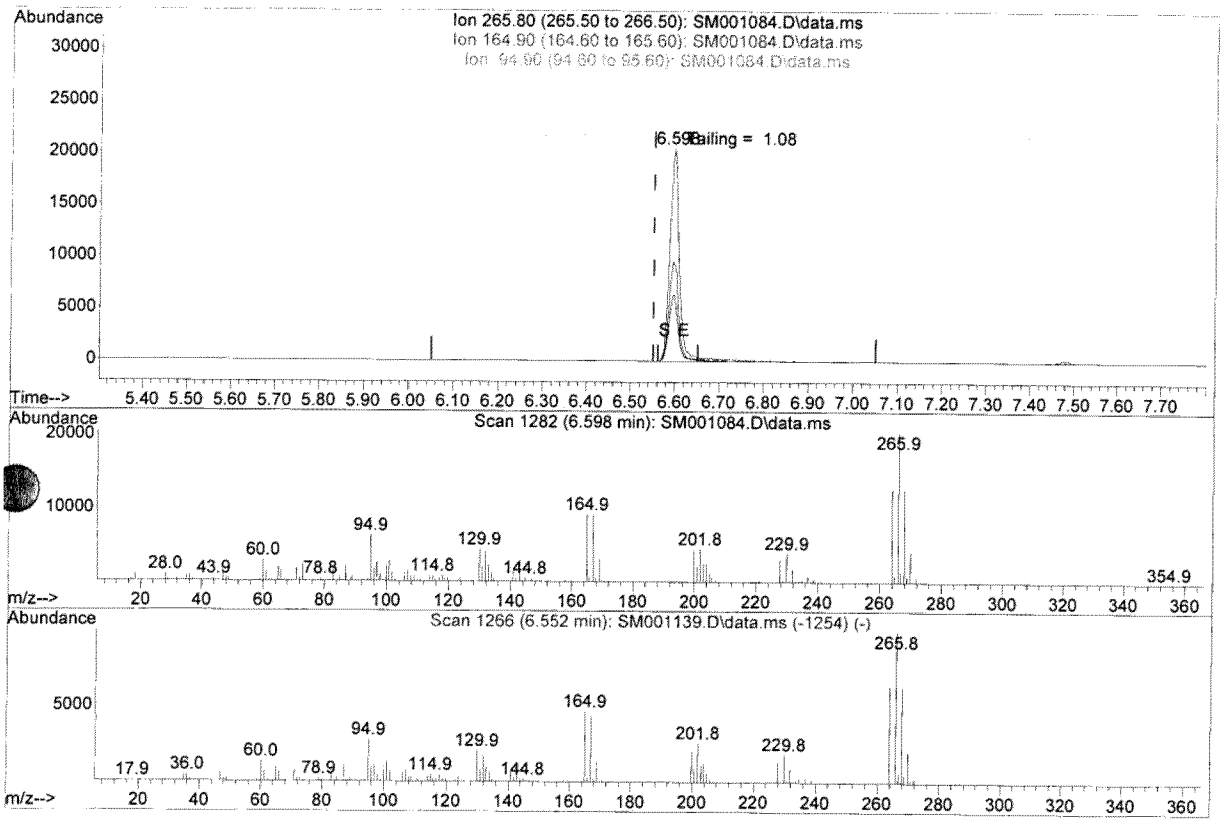


TAILING FACTOR= $\frac{BC}{AB}$

Example calculation: Peak Height = DE = 100 mm
10% Peak Height = BD = 10 mm
Peak Width at 10% Peak Height = AC = 23 mm
AB = 11 mm
BC = 12 mm

Therefore: Tailing Factor = $\frac{12}{11}$ = 1.1

FIGURE 2. Tailing factor for pentachlorophenol and benzidine, and the % breakdown of DDT calculated using the HP Enviroquant software



TIC: SM001084.D\data.ms

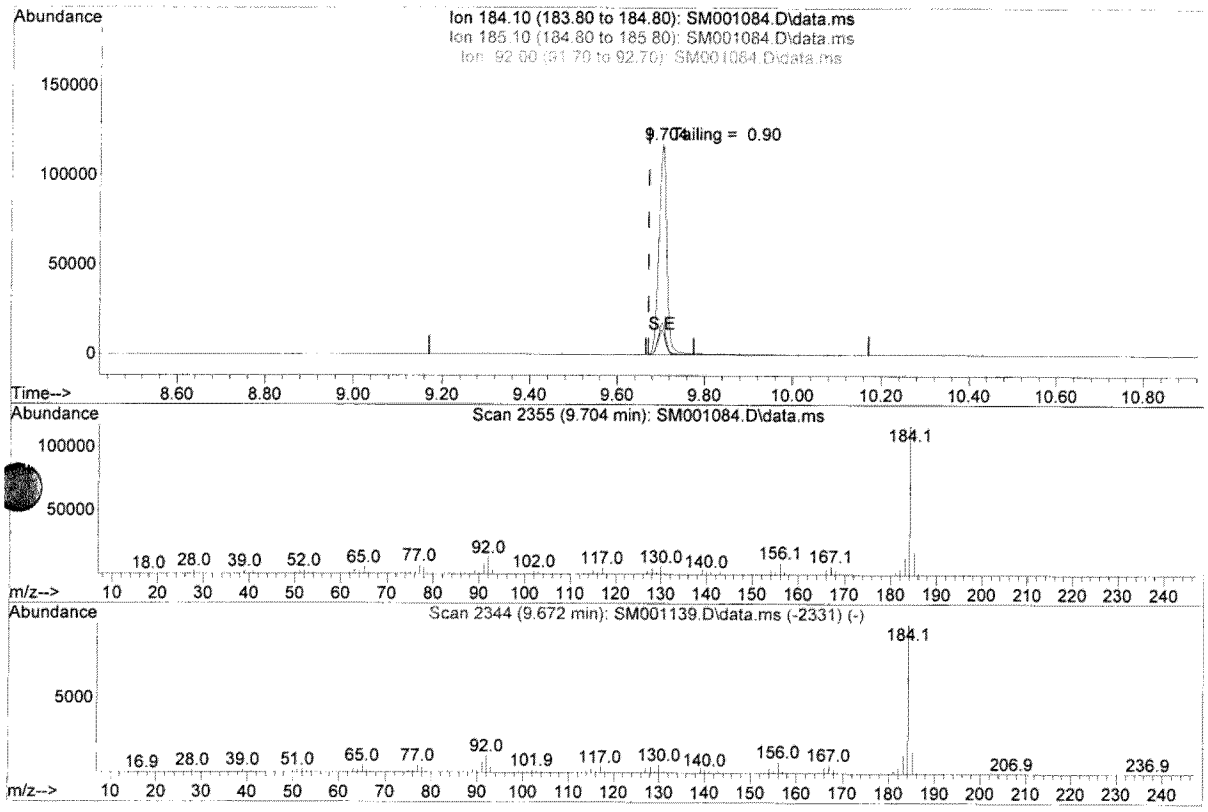
(4) Pentachlorophenol

6.598min (+0.046) 39.83 ug/mL m

response 31004

Ion	Exp%	Act%
265.80	100	100
164.90	46.20	46.02
94.90	27.90	29.54
0.00	0.00	0.00

FIGURE 2. Tailing factor for pentachlorophenol and benzidine, and the % breakdown of DDT calculated using the HP Enviroquant software (cont'd)



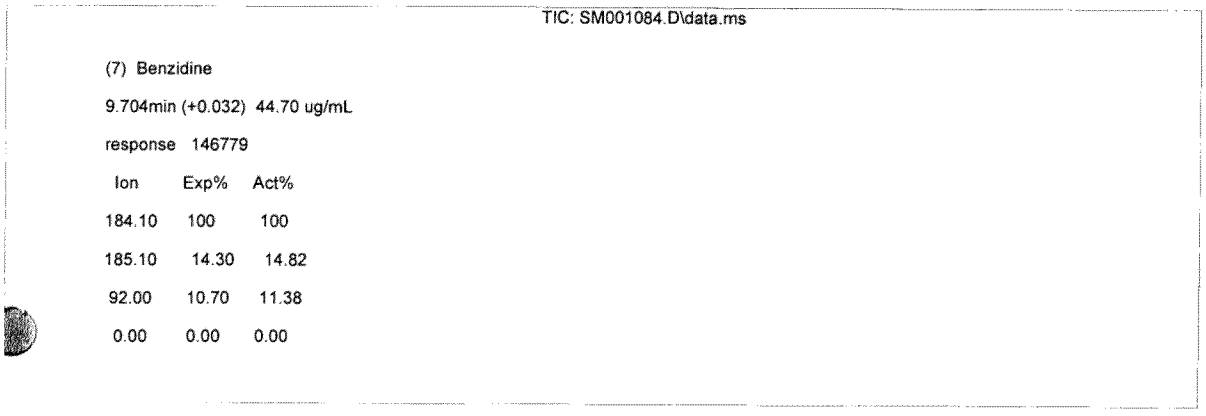
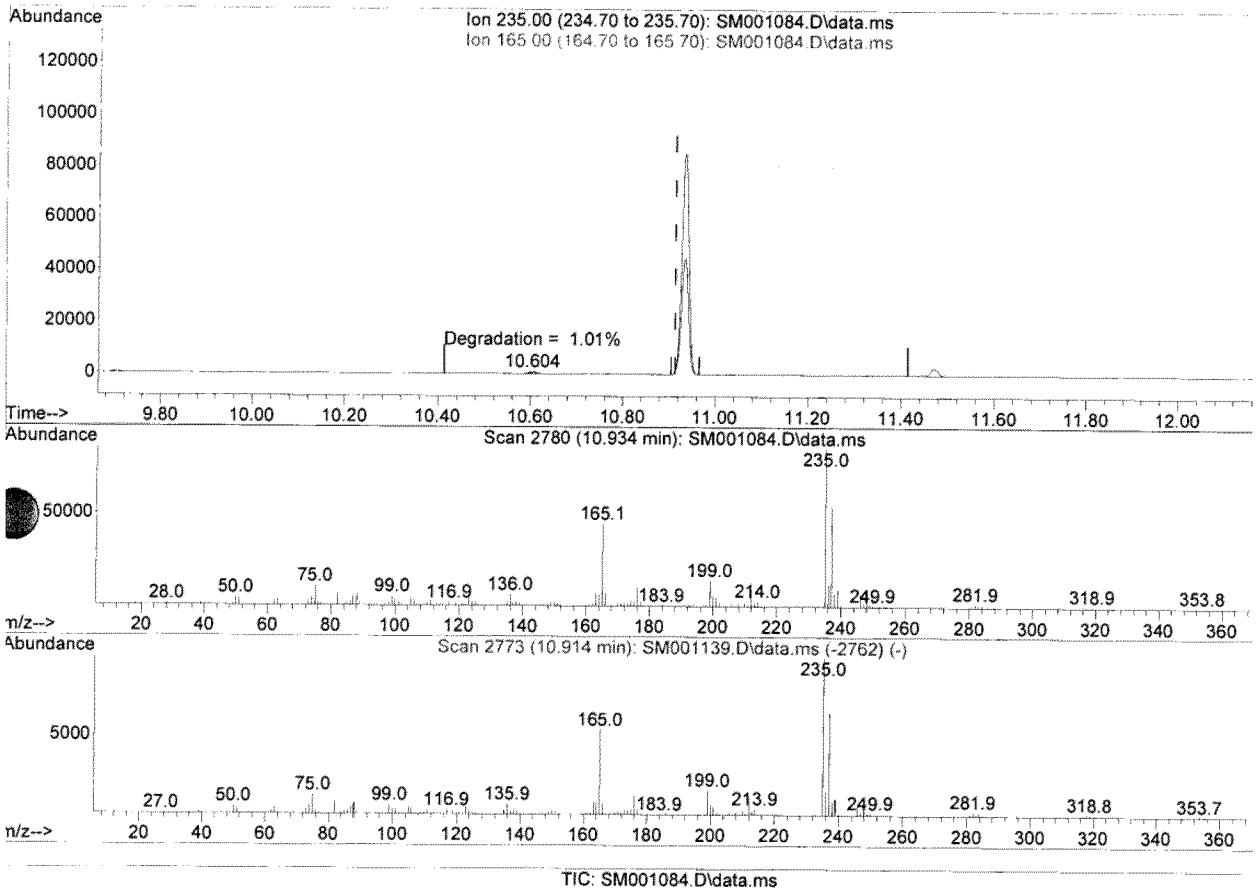


FIGURE 2. Tailing factor for pentachlorophenol and benzidine, and the % breakdown of DDT calculated using the HP Enviroquant software (cont'd)



(8) DDT

10.934min (+0.020) 52.85 ug/mL

response 90296

Ion	Exp%	Act%
235.00	100	100
165.00	52.10	53.15
0.00	0.00	0.00
0.00	0.00	0.00