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Title: Organophosphorus Pesticides by Gas Chromatography [Methods 8141A, 8141B and 614]

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1.0 Scope and Application

1.1 This method is applicable to the determination of the concentration of certain organophosphorus pesticides in extracts of waters, waste waters, oils, soils, and sludges. It is based on SW-846 Method 8141A, 8141B and EPA Method 614.

1.2 Table 1 shows reporting limits for compounds routinely analyzed by this method. The compounds that can be analyzed by this SOP include, but are not limited to, those shown in Table 1.

2.0 Summary of Method

2.1 Instructions for sample preparation are outside of the scope of this SOP. Instructions for the extraction of aqueous samples using a separatory funnel by SW-846 Method 3510C are provided in SOP DV-OP-0006. Instructions for preparing solid samples using SW-846 Method 3540C Soxhlet extraction are provided in SOP DV-OP-0010. Instructions for concentrating organic extracts are provided in SOP DV-OP-0007.

2.1.1 A one-liter portion of an aqueous sample is extracted using methylene chloride and solvent exchanged to hexane with a final volume of 2 mL.

2.1.2 A 30-gram portion of a soil sample is extracted using a 1:1 mixture of methylene chloride and acetone. The extract is solvent exchanged to hexane with a final volume of 2 mL.

2.2 The sample extract is introduced into a dual column gas chromatograph (GC) equipped with nitrogen-phosphorous detectors (NPD). The concentrations of target analytes are determined on the primary column by comparing chromatographic peaks at predetermined retention times to the same chromatographic peaks for calibration standards. The internal standard method of calibration is used, and target analyte confirmation is made using a second dissimilar capillary column.

3.0 Definitions

Refer to the Denver Quality Assurance Manual and SOP DV-QA-003P for definitions of QA/QC terms used in this document.

4.0 Interferences

4.1 Florisil clean-up procedures are not recommended due to poor recoveries.

4.2 Many organophosphorous pesticides will degrade on active sites in the chromatographic system. Therefore, injection port maintenance is critical. Regular replacement of the glass liner, glass wool, septum, gold seal, and clipping of the guard column should be performed.

4.3 Retention times of some analytes may increase with increasing concentrations in the injector. Retention time shifts must be monitored and evaluated, especially for highly contaminated samples.

4.4 Triazine herbicides, such as atrazine and simazine, and other nitrogen-containing compounds may interfere when using a nitrogen-phosphorus detector.

4.5 Tetraethyl pyrophosphate (TEPP) is an unstable diphosphate that is readily hydrolyzed in water and decomposes at 170 °C. Methyl parathion is also readily hydrolyzed.

4.6 The water solubility of dichlorvos (DDVP) is 10 g/L at 20 °C, and recovery is poor from aqueous samples.

4.7 Naled can be converted to dichlorvos (DDVP) by debromination; and this reaction may occur during sample work-up or on column.

4.8 Demeton is a mixture of two isomers, demeton-S and demeton-O. Two peaks are observed in all chromatograms corresponding to these two compounds. Depending on project requirements, results may be reported in terms of individual components and/or total demeton.

4.9 Merphos is a single component pesticide that is readily oxidized to merphos oxone. Chromatographic analysis of merphos almost always results in two peaks. As the relative amounts of oxidation of the sample and the calibration standard are probably different, quantitation of this compound is performed by summation of the area of peaks.

4.10 Mevinphos is a mixture of cis and trans isomers. Both isomers are combined for quantitation of mevinphos.

5.0 Safety

5.1 Employees must abide by the policies and procedures in the Environmental Health and Safety Manual, Radiation Safety Manual, and this document.

This procedure may involve hazardous material, operations and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous. Safety glasses, gloves, lab coats and closed-toe, nonabsorbent shoes are a minimum.

5.2 Specific Safety Concerns or Requirements

5.2.1 Eye protection that satisfies ANSI Z87.1, laboratory coat, and nitrile gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated must be removed and discarded; non-disposable gloves must be cleaned immediately.

5.2.2 The gas chromatograph contains zones that have elevated temperatures. The analyst needs to be aware of the locations of those zones, and must cool them to room temperature prior to working on them.

5.2.3 There are areas of high voltage in the gas chromatograph. Depending on the type of work involved, either turn the power to the instrument off, or disconnect it from its source of power.

5.3 Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating.

NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the MSDS for each of the materials listed in the table.

A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the MSDS for each material before using it for the first time or when there are major changes to the MSDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and Symptoms of Exposure
Acetone	Flammable	1000 ppm (TWA)	Inhalation of vapors irritates the respiratory tract. May cause coughing, dizziness, dullness, and headache.
Hexane	Flammable Irritant	500 ppm (TWA)	Inhalation of vapors irritates the respiratory tract. Overexposure may cause lightheadedness, nausea, headache, and blurred vision. Vapors may cause irritation to the skin and eyes.
Methanol	Flammable Poison Irritant	200 ppm (TWA)	A slight irritant to the mucous membranes. Toxic effects exerted upon nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness and dizziness. Methyl alcohol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms may parallel inhalation exposure. Irritant to the eyes.
Organo-phosphorus Pesticide Standards	Toxic		In almost all cases, the pesticide standards are purchased as stock standards made up in one of the solvents listed in this table and the hazards associated with the standard solutions are primarily those of the solvent. Exposure to organophosphorus pesticides produces effects on the nervous system. Acute exposure compromises neuromuscular functions, decreases motor activity and body temperature, alters cardiovascular function, and can also have a delayed neuromuscular effect that is irreversible and leads to paralysis.
(1) Always add acid to water to prevent violent reactions. (2) Exposure limit refers to the OSHA regulatory exposure limit.			

6.0 Equipment and Supplies

6.1 An analytical system consisting of a dual column gas chromatograph and nitrogen-phosphorus detectors (NPD). A data system capable of measuring peak area and/or height.

6.1.1 Proper conditioning of the NPD bead is essential to obtain acceptable performance. Lowering the voltage level during periods of non-use may extend bead life.

6.1.2 Suggested columns: Rtx®-OPP (30 m X 0.32 mm X 0.5 μm), Rtx®-1MS (30 m X 0.32 mm X 0.25 μm), or Rtx®-OPP2 (30 m X 0.32 mm X 0.5 μm).

6.1.3 See Table 2 for suggested instrument operating conditions.

6.2 Microsyringes, various sizes, for standards preparation, sample injection, and extract dilution. Various class A volumetric flasks for standard preparation.

6.3 Computer Software and Hardware

6.3.1 Please refer to the master list of documents, software, and hardware located on G:\QA\Read\Master List of Documents\Master List of Documents, Software and Hardware.xls or current revision for the current software and hardware to be used for data processing.

7.0 Reagents and Standards

7.1 Stock Standards

7.1.1 Standards are purchased commercially and are received as certified solutions in flame-sealed ampoules.

NOTE: The availability of the specific commercial standard solutions upon which the following sections are based may change at any time. As a result, it may be necessary to alter the dilution scheme presented herein to accommodate changes in stock standard concentrations. All such changes are documented in the standards preparation records.

7.1.2 Standards are verified before use as described in SOP DV-QA-0015.

7.1.3 Stock standards are stored refrigerated at $\leq 6\ ^{o}C$, or as recommended by the manufacturer. All stock standards must be protected from light.

7.1.4 Stock standard solutions should be brought to room temperature before using.

7.1.5 Stock standards are monitored for signs of degradation or evaporation. The standards must be replaced annually from the date of receipt or earlier if the vendor indicates an earlier date.

7.1.6 Typical certified stock standards for this method include the following:

7.1.6.1 Restek (32277), 8140/8141 OP Pesticides Calibration Mix A (primary source) – 200 μg/mL

7.1.6.2 Restek (563721), Custom 8141 OPP Additions Standard (primary source) – 200 μg/mL

7.1.6.3 ChemService (MET-88A), Carbophenothion methyl (primary source) – 100 μg/mL

7.1.6.4 AccuStandard (M-8140M-5x), 8140 Organo Phosphorous Pesticides Calibration Mix A (second source, LCS/MS)– 200 μg/mL

7.1.6.5 AccuStandard (S-18291-R2), 8141 Custom Spike Standard (second source, LCS/MS) – 200 μg/mL

7.1.6.6 AccuStandard (P-652S), Methyl Trithion (second source, LCS/MS) – 100 μg/mL

7.1.6.7 AccuStandard (P-329S), Chlormephos (surrogate) – 1000 μg/mL

7.1.6.8 AccuStandard (P-192S-10x), Triphenyl Phosphate (surrogate) – 1000 μg/mL

7.1.6.9 Restek (32280), Tributyl phosphate (internal standard) – 1000 μg/mL

7.1.6.10 ChemService (F2425S), Tri-o-cresyl phosphate (internal standard) – 1000 μg/mL

7.1.6.11 Supelco (NQ21184312) custom 8141 mix (second source) 200 ug/mL.

7.2 Non-Routine Compounds

Other, non-routine compounds not listed in this section may be requested by a client and may be added to this procedure.

7.2.1 In these cases, all stock solutions will be obtained from commercial sources and will be verified with a second-source standard as described in Section 7.1 above.

7.2.2 Non-routine standards will be stored and treated as described in Section 7.1 above.

7.2.3 Subsequent dilutions of specially requested compounds will be determined in a manner consistent with the client's recommendations for number of calibration points, inclusion of reporting limit, and concentration range adequate to represent the linearity of the instrument.

7.2.4 These specially requested, non-routine compounds either may be added to the dilution scheme used for routine compounds or may be prepared as a separate calibration.

7.2.5 All standards preparation for non-routine compounds shall be documented using the same method that is used for routine compounds.

7.3 Secondary Standards, 5 μg/mL

7.3.1 Dilutions from stock standards are made in pesticide-grade hexane and acetone (90:10) solvent mixture because some certified stock standards are prepared in methanol. The Supelco second source custom 8141 mix (NQ21184312) is diluted in acetone.

7.3.2 A secondary standard dilution is prepared at a concentration of 5 μg/mL, and further dilutions of this standard are made for calibration. This secondary standard should be monitored for degradation and prepared every 2 months. Particular attention should be made to check methyl parathion and merphos monthly. This standard cannot have a later expiration date than the date assigned to the parent stock solutions. See Table 3 for specifications.

7.3.3 All standards must be refrigerated at $\leq$ 6 ^{o}C and protected from light.

7.4 Calibration Standards

Calibration standards are prepared as dilutions of the secondary standard at the levels shown in Table 3.

7.5 RL Standard

The Level 1 calibration standard (see Table 3) is typically used as the RL Standard.

7.6 Second-Source Initial Calibration Verification Standard, 2.0 μg/mL

Two initial calibration verification standards are prepared from a different source than the calibration standards themselves. If a second comparable manufacturer stock standard is not available, then the second-source standard is prepared from the same stock by a different analyst. The calibration verification is typically prepared at a concentration of 2.0 μg/mL and 5 ug/mL. See Table 3 for the exact concentrations of each analyte in each of the ICV dilutions.

7.7 Laboratory Control Standard (LCS, same as QCS) and Matrix Spike Standards.

The standard spiking solution is prepared from the second source material listed in 7.1.6 diluted with acetone. See Table 4 for the concentrations and list of target constituents. The solution is prepared by diluting 1 mL of S-18291-R2, 1 mL of M8140M-5x, and 2 mL of P652S to a final volume of 50 mL. (See Section 7.1.6 for standard solution identifications.)

7.8 Surrogate Standards

Chlormefos and triphenyl phosphate are the surrogate standards. Refer to Table 4 for details of surrogate standards. The surrogate solution is prepared at 2ug/mL by diluting 0.5 mL of P-329S-10x and 0.1 mL of M-507-1S-10x to a final volume of 250 mL.

7.9 Internal Standard (IS) Solution, 100 μg/mL

An internal standard solution is prepared containing tributyl phosphate and tri-o-cresylphosphate at a final concentration of 100 μg/mL in hexane. The IS solution is prepared by combining 1 mL of each of these solutions listed in 7.1.6 and diluting to 10 mL in Hexane:Acetone (90:10). The IS is added to each calibration standard, calibration verification standard, field sample extract, and QC sample extract that is analyzed. The internal standard is added to the extract just prior to analysis.

7.10 Reagents

7.10.1 Hexane, pesticide grade

7.10.2 Acetone, pesticide grade

7.10.3 Helium gas, ≥ 99.99999% pure

7.10.4 Hydrogen gas, ≥ 99.99999% pure

8.0 Sample Collection, Preservation, Shipment and Storage

8.1 Samples must be collected in pre-cleaned amber glass bottles fitted with a Teflon-lined cap. To achieve routine reporting limits, a full one-liter of sample is required. Additional one-liter portions are needed to satisfy the requirements for matrix spikes and duplicate matrix spikes.

8.2 The pH of aqueous samples is to be checked upon arrival at the lab and adjusted to a pH of 5 to 8 using sodium hydroxide or sulfuric acid as soon as possible.

8.3 Samples are stored at $\leq$ 6 °C.

8.4 Extracts must be refrigerated at $\leq$ 6 °C.

Matrix	Sample Container	Min. Sample Size	Preservation	Extraction Holding Time	Analysis Holding Time	Reference
Waters	Amber glass	1 Liter	Cool $\leq$ 6 °C	7 Days	40 Days from extraction	SW-846 40 CFR Part 136
Soils	Glass	30 grams	Cool $\leq$ 6 °C	14 Days (8141A) 7 Days (8141B)	40 Days from extraction	SW-846

9.0 Quality Control

9.1 The minimum quality controls (QC), acceptance criteria, and corrective actions are described in this section. When processing samples in the laboratory, use the LIMS Method Comments to determine specific QC requirements that apply.

9.1.1 The laboratory's standard QC requirements, the process of establishing control limits, and the use of control charts are described more completely in TestAmerica Denver policy DV-QA-003P, Quality Assurance Program.

9.1.2 Specific QC requirements for Federal programs, e.g., Department of Defense (DoD), Department of Energy (DOE), AFCEE, etc., are described in TestAmerica Denver policy DV-QA-024P, Requirements for Federal Programs.

9.1.3 Project-specific requirements can override the requirements presented in this section when there is a written agreement between the laboratory and the client, and the source of those requirements should be described in the project documents. Project-specific requirements are communicated to the analyst via Method Comments in the LIMS and the Quality Assurance Summaries (QAS) in the public folders.

9.1.4 Any QC result that fails to meet control criteria must be documented in a Nonconformance Memo (NCM). The NCM is automatically sent to the laboratory Project Manager by e-mail so that the client can be notified as appropriate. The QA group periodically reviews NCMs for potential trends. The NCM process is described in more detail in SOP DV-QA-0031. This is in addition to the corrective actions described in the following sections.

9.2 Initial Performance Studies

Before analyzing samples, the laboratory must establish a method detection limit (MDL). In addition, an initial demonstration of capability (IDOC) must be performed by each

analyst. On-going proficiency must be demonstrated by each analyst on an annual basis. See Section 13 for more details on detection limit studies, initial demonstrations of capability, and analyst training and qualification.

9.3 Batch Definition

Batches are defined at the sample preparation stage. The batch is a set of up to 20 field samples of the same matrix, plus required QC samples, processed using the same procedures and reagents within the same time period. Batches should be kept together through the whole analytical process as far as possible, but it is not mandatory to analyze prepared extracts on the same instrument or in the same sequence except the method blank must be analyzed on each instrument used to analyze samples from the same preparation batch. See policy DV-QA-003P for further details.

9.4 Method Blank

Each batch of samples must include a method blank. For aqueous sample batches, the method blank consists of reagent water with the addition of surrogate spike compounds. For soil sample batches, the method blank consists of Ottawa sand spiked with the surrogate compounds. The method blank is subject to the entire extraction and analysis process.

Acceptance Criteria: The method blank must not contain any analyte of interest at or above 1/2 the reporting limit or above one-tenth of the concentration found in the associated samples.

Corrective Action: If the method blank exceeds allowable levels, all associated samples that have detections for targeted compounds must be re-extracted and reanalyzed.

9.5 Quality Control Check Sample (QCS) / Laboratory Control Sample (LCS)

The LCS contains representative analytes of interest, as well as the surrogate compounds, added to reagent water or Ottawa sand, depending on the sample matrix. The LCS is subject to the entire extraction and analysis process.

Acceptance Criteria: The percent recovery of each analyte of interest must fall within the established control limits. Control limits are set at $\pm$ 3 standard deviations around the historical mean, unless otherwise dictated by project requirements.

When there are more than 11 target analytes in the LCS, then NELAC allows a specified number of results to fall beyond the LCS control limit (3 standard deviations), but within the marginal exceedance (ME) limits, which are set at $\pm$ 4 standard deviations around the mean of historical data. The number of marginal exceedances is based on the number of analytes in the LCS, as shown in the following table:

Allowed Marginal Exceedances

# of Analytes in LCS	# of Allowed MEs
> 90	5

# of Analytes in LCS	# of Allowed MEs
71 – 90	4
51 – 70	3
31 – 50	2
11 – 30	1
< 11	0

If more analytes exceed the LCS control limits than is allowed, or if any analyte exceeds the ME limits, the LCS fails and corrective action is necessary. Marginal exceedances must be random. If the same analyte repeatedly fails the LCS control limits, it is an indication of a systematic problem. The source of the error must be identified and corrective action taken.

Note: Some programs (e.g., South Carolina) do not allow marginal exceedences. Please see the QAS's in the public folders for the current requirements.

Corrective Action: If recoveries are not within the acceptance limits as defined above, the system is out of control and corrective action must occur. All associated samples must be re-extracted and reanalyzed. If recoveries are high-biased and the associated samples are ND, then the data may be reported with narration in an NCM.

9.6 Matrix Spike and Matrix Spiked Duplicate (MS/MSD)

At a minimum, the laboratory must prepare one MS/MSD pair in every batch of 20 samples. If there is insufficient volume to prepare a MS/MSD pair, a LCS/LCSD must be prepared to provide precision data for the batch.

For method 614, the laboratory must spike one sample in every batch and spike 10% of all samples. If the batch has more than 10 samples, then two matrix spikes must be performed. The two matrix spikes are to be performed on two different samples.

An MS is prepared by adding representative analytes of interest and surrogate compounds to an aliquot of a designated field sample. The MSD is prepared by adding the same analytes and surrogates to a second aliquot of the designated sample.

Acceptance Criteria: The percent recovery of each analyte of interest must fall within the established control limits. Control limits are set at $\pm$ 3 standard deviations around the historical mean, unless otherwise dictated by project requirements.

Corrective Actions: If any individual recovery or RPD falls outside the acceptable range, corrective action must occur. The initial corrective action will be to check the recovery of that analyte in the LCS. Generally, if the recovery of the analyte in the LCS is within limits, then the laboratory operation is in control and analysis may proceed.

If the recovery for any component is outside QC limits for both the MS/MSD and the LCS, the laboratory is out of control and corrective action must be taken. Corrective action will normally include re-extraction and reanalysis of the batch.

If an MS/MSD pair is not possible due to limited sample, then a duplicate LCS may be analyzed, depending on project requirements.

The MS and MSD must be analyzed at the same dilution level as the unspiked sample, unless the matrix spike components would then be above the calibration range.

9.7 Surrogates

The surrogate compounds chlormefos and triphenyl phosphate are added to all field and QC samples. Refer to Section 7.8 for preparation of the surrogate spike solution. Surrogate recoveries must be assessed to ensure that analytical recoveries are within established limits.

Acceptance Criteria: The percent recovery of each analyte of interest must fall within the established control limits. Control limits are set at $\pm$ 3 standard deviations around the historical mean, unless otherwise dictated by project requirements.

Corrective Action: Check all calculations for error.

Ensure that instrument performance is acceptable.

Recalculate the data and/or reanalyze the extract if either of the above checks reveals a problem.

Re-extract and reanalyze the sample or flag the data as "Estimated Concentration" if neither of the above resolves the problem. Re-extraction may not be necessary if there is obvious and well documented chromatographic interference.

The decision to reanalyze or flag the data should be made in consultation with the client. It is only necessary to re-extract / reanalyze a sample once to demonstrate that poor surrogate recovery is due to matrix effect, unless the analyst believes that the repeated out of control results are not due to matrix effect.

9.8 Internal Standard

Internal standard compounds are added to all field and QC sample extracts. Internal standard compound recoveries must be assessed to ensure that analytical recoveries are within established limits.

Acceptance Criteria: Method 8141B requires that the measured area of the internal standard must be no more than 50% different from the average area calculated during calibration. For other analyses (Method 8141A or Method 614) the measured area of the internal standard

must be no more than 200% and no less than 50% different from the average area calculated during calibration.

Corrective Action: Check all calculations for error.

Ensure that instrument performance is acceptable.

Recalculate the data and/or reanalyze the extract if either of the above checks reveals a problem. Where interferences are indicated reanalysis at a dilution may be required to resolve this issue.

9.9 2012 MUR Required QC Elements

The May 2012 EPA Method Update Rule (MUR) to 40 CFR Part 136 for compliance testing under the Clean Water Act (CWA) requires laboratories to include 12 QC elements when performing the published or approved methods. See Work Instruction WI-DV-0060, QC Requirements for Methods Designated in 40 CFR Part 136, for list of approved test procedures performed by TestAmerica-Denver and the required QC elements in each of these methods.

10.0 Calibration and Standardization

10.1 TestAmerica Denver gas chromatograph instrument systems are computer controlled to automatically inject samples and process the resulting data.

10.1.1 Detailed information regarding calibration models and calculations can be found in Corporate SOP CA-Q-S-005, *Calibration Curves (General)* and under the public folder, Arizona Calibration Training.

10.1.2 Use the ChemStation chromatography data system to set up GC conditions for calibration. See Table 2 for typical operating conditions.

10.1.3 Transfer calibration standard solutions into autosampler vials and load into the GC autosampler. Use the ChemStation software to set up the analytical sequence.

10.1.4 Unprocessed calibration data are transferred to the TARGET DB database for processing. After processing the calibration data, print the calibration report and review it using the calibration review checklist (GC and HPLC Data Review Checklist - ICAL). Submit the calibration report to a qualified peer or the group leader for final review. The completed calibration reports are scanned and stored as Adobe Acrobat files on the Pubic Drive.

10.2 The laboratory routinely calibrates using seven concentration levels. A minimum of five calibration levels are required (six if a second order regression fit is used). The lowest point on the calibration curve is below the RL, and is close to the MDL concentrations. These concentrations define the working range for analysis.

NOTE: Generally, it is **NOT** acceptable to remove points from a calibration for the purposes of meeting calibration criteria. If calibration acceptance criteria are not met, the normal corrective action is to examine conditions such as instrument maintenance and accuracy of calibration standards. Any problems found must be fixed and documented in the run log or maintenance log. Then the calibration standards must be reanalyzed.

10.3 If no problems are found or there is documented evidence of a problem with a calibration point (e.g., obvious mis-injection or broken vial), then one point might be rejected but only if all of the following conditions are met:

10.3.1 The rejected point is the highest or lowest on the curve, i.e., the remaining points used for calibration must be contiguous; and

10.3.2 The lowest remaining calibration point is still at or below the project reporting limit; and

10.3.3 The highest remaining calibration point defines the upper concentration of the working range, and all samples producing results above this concentration are diluted and reanalyzed; and

10.3.4 The calibration must still have a minimum number of calibration levels required by the method, i.e., five levels for calibrations modeled with average calibration factors or linear regressions, or six levels for second order curve fits.

Note: Second order curve curves are not allowed for South Carolina work.

10.4 All initial calibration points must be analyzed without any changes to **instrument conditions and all points must be analyzed within 24 hours.**

10.5 Two internal standards (tri-o-cresyl phosphate and tributyl phosphate) are used for calibration. The area of the chromatographic peak for each analyte of interest is used as the instrument response. If matrix interferences would make quantitation using peak area inaccurate for a particular sample, then peak height may be used as a substitute, but it would then have to be used consistently for all files and QC samples analyzed in the analytical batch.

10.6 Internal Standard Calibration

Internal standard calibration involves the comparison of an instrument response (e.g., peak area or peak height) from the target compound in the sample to the response of the internal standard compound, which is added to the sample or sample extract prior to injection. For this method, tributyl phosphate and tri-o-cresylphosphate are added to the sample and QC extracts just prior to analysis at a concentration level of 2.0 µg/mL. This same concentration of internal standard is added to each initial calibration standard.

10.7 Establishing the Calibration Function

Calibrations are modeled either as average response factors or as calibration curves, using a systematic approach to selecting the optimum calibration function. Start with the simplest model, i.e., a straight line through the origin and progress through the other options until the calibration acceptance criteria are met.

10.7.1 Linear Calibration Using Average Response Factor

A calibration curve relates the instrument response, usually in terms of area of the chromatographic peak, to the concentration of the target analyte in each of the calibration standards. If linearity through the origin can be demonstrated, then the average response factor can be used to calculate the target analyte concentration in an unknown sample. The response factor is a measure of the slope of the

calibration line, assuming that the line passes through the origin. Under ideal conditions, the factors calculated for each calibration level will not vary with the concentration of the standard. In practice, some variation can be expected. When the variation, measured as the relative standard deviation, is relatively small, the use of the straight line through the origin model is generally appropriate. The response factor is calculated as the ratio of the area response of the target analyte to the area response of the internal standard, as follows:

$$RF_i = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$ *Equation* 1

Where:

RF_i = Response factor for the i[th] calibration level.

A_s = Area of chromatographic peak for the analyte of interest in the calibration standard.

A_{is} = Area of chromatographic peak for the internal standard.

C_{is} = Concentration of the internal standard, μg/mL.

C_s = Concentration of the analyte of interest in the calibration standard, μg/mL.

10.7.2 The average response factor and its associated relative standard deviation (RSD) are calculated as follows:

$$\overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n}$$ *Equation 2*

$$SD = \sqrt{\frac{\sum_{i=1}^{n} \left(RF_i - \overline{RF}\right)^2}{n-1}}$$ *Equation 3*

$$\%RSD = \frac{SD}{\overline{RF}} \times 100$$ *Equation 4*

Where:

$\overline{RF}$ = Average response factor.

RF_i = Response *factor* for the i[th] calibration level.

n = Number of calibration levels.

SD = Standard deviation.

%RSD = Relative standard deviation, expressed as a percent.

10.7.3 Evaluation of the Average Response Calibration

Plot the calibration curve using the average RF as the slope of a line that passes through the origin. Examine the residuals, i.e., the difference between the actual calibration points and the plotted line. Particular attention should be paid to the

residuals for the highest points, and if the residual values are relatively large, a linear regression should be considered.

Acceptance Criteria: For Method 614, the %RSD must be ≤ 10%.
For Method 8141A/8141B, the %RSD must be ≤ 20%.

Corrective Action: If the RSD exceeds the limit, linearity through the origin cannot be assumed, and a least-squares linear regression should be attempted.

10.7.4 Linear Calibration Using Least-Squares Regression

Calibration using least-squares linear regression produces a straight line that does not pass through the origin. The calibration relationship is constructed by performing a linear regression of the instrument response (peak area or peak height) versus the concentration of the standards. The instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). The weighting used is the reciprocal of the concentration or the reciprocal of the square of the concentration. The regression produces the slope and intercept term for a linear equation in the following form:

$$y = ax + b \qquad \textit{Equation 5}$$

Where:

- y = Instrument response (peak area or height).
- x = Concentration of the target analyte in the calibration standard.
- a = Slope of the line.
- b = The y-intercept of the line.

For an internal standard calibration, the above equation takes the following form:

$$\frac{A_s C_{is}}{A_{is}} = aC_s + b \qquad \textit{Equation 6}$$

To calculate the concentration in an unknown sample extract, the regression equations 5 and 6 are solved for concentration, resulting in the following equations, where x and C_s are now the concentration of the target analyte in the unknown sample extract:

$$x = \frac{y - b}{a} \qquad \textit{Equation 7}$$

$$C_s = \frac{\left[\frac{A_s C_{is}}{A_{is}} - b\right]}{a} \qquad \textit{Equation 8}$$

10.7.5 Evaluation of the Linear Least-Squares Regression Calibration Function

With an unweighted linear regression, points at the lower end of the calibration curve have less weight in determining the curve than points at the high

concentration end of the curve. For this reason, inverse weighting of the linear function is recommended to optimize the accuracy at low concentrations. Note that the August 7, 1998 EPA memorandum "Clarification Regarding Use of SW-846 Methods", Attachment 2, Page 9, includes the statement "The Agency further recommends the use of this for weighted regression over the use of an unweighted regression."

Acceptance Criteria: To avoid bias in low level results, the absolute value of the y-intercept must be significantly less than reporting limit (RL), and preferably less than the MDL.

Also examine the residuals, but with particular attention to the residuals at the low end of the curve. If the intercept or the residuals are large, a second-order regression should be considered.

The linear regression must have a correlation coefficient (r) ≥ 0.990. Some programs (e.g., DoD, USACE and AFCEE) require a correlation coefficient (r) ≥ 0.995.

Corrective Action: If the correlation coefficient falls below the acceptance limit, linear regression cannot be used and a second-order regression should be attempted.

10.7.6 Non-Linear Calibration

When the instrument response does not follow a linear model over a sufficiently wide working range, or when the previously described calibration approaches fail acceptance criteria, a non-linear, second-order calibration model may be employed. The second-order calibration uses the following equation:

$$y = ax^2 + bx + c \qquad \textit{Equation 9}$$

Where a, b, and c are coefficients determined using a statistical regression technique; y is the instrument response; and x is the concentration of the target analyte in the calibration standard.

10.7.7 Evaluation of Second-Order Regression Calibration

A minimum of six points must be used for a second-order regression fit.

Acceptance Criteria: The coefficient of determination (COD) must be (r^2) ≥ 0.990.

Second-order regressions should be the last option, and note that some programs (e.g., South Carolina) do not allow the use of second-order regressions. A second order model must not be used to avoid maintenance. Before selecting a second-order regression calibration model, it is important to ensure the following:

- The absolute value of the intercept is approximately < one-half of the lowest concentration standard reported.

- The response increases significantly with increasing standard concentration (i.e., the instrument response does not plateau at high concentrations).
- The distribution of concentrations is adequate to characterize the curvature.

Corrective Action: If the coefficient of determination falls below the acceptance limit and the other calibration models are unacceptable, the source of the problem should be investigated and the instrument recalibrated.

IMPORTANT: Third-order regressions are not allowed at TestAmerica Denver.

10.8 Second-Source Initial Calibration Verification (ICV)

A mid-level standard that is obtained from a source different from that of the calibration standards is used to verify the initial calibration (see Section 7.6). The ICV is analyzed immediately following the initial calibration (ICAL) at two different concentrations.

Acceptance Criteria: The result for any single target analyte in the ICV standard should be within ± 15% of the expected value. Method 8141B allows for ± 20%. However, the analysis is acceptable if the average of the percent difference (%D) values for all the analytes is within ± 15% and the %D for all individual analytes is within ± 30% (per Method 8000B).

Note: The grand mean of the %D is not allowed in Method 8000C.

Note: Anilazine and Naled are documented poor performers and are allowed a %D of ± 50%.

Corrective Action: If this is not achieved, the ICV standard, calibration standards, and instrument operating conditions should be checked. Correct any problems and rerun the ICV standard. If the ICV still fails to meet acceptance criteria, then recalibrate the instrument.

10.9 Calibration Verification

10.9.1 12-Hour Calibration Verification

The 12-hour calibration verification sequence consists of, at a minimum, an instrument blank and the mid-level calibration standard. The 12-hour calibration verification sequence must be analyzed within 12 hours of the initial calibration and at least once every 12 hours thereafter when samples are being analyzed.

NOTE: It is not necessary to run a CCV standard at the beginning of the sequence if samples are analyzed immediately after the completion of the initial calibration.

10.9.2 Continuing Calibration Verification (CCV)

It may be appropriate to analyze a mid-level standard more frequently than every 12 hours. The mid-level calibration standard is analyzed as the continuing calibration verification (CCV) standard (see Table 3). At a minimum, this is analyzed after every 20 samples, including matrix spikes, LCSs, and method blanks. Some programs, specifically drinking water programs, require a CCV after every 10 samples to minimize the number of samples requiring re-analysis when QC limits are exceeded. If 12 hours elapse, analyze the 12-hour standard sequence instead. As recommended by method 8000, some programs, (e.g., Wisconsin and Arizona) require that any compound that uses a second order regression be checked with CCVs at two concentration levels, a midpoint and one near the RL. Check method comments and QASs in the public folders to verify project requirements for the two level verification.

10.9.3 RL Standard

It may also be appropriate to analyze a standard prepared at or very near the reporting limit (RL) for the method at the end of the analytical sequence (see Section 7.5). This standard can be used to rule out false negatives in client samples in cases where the %D for one or more of the analytes in a bracketing CCV falls below the lower acceptance limit. The results for the RL standard are not evaluated **unless** the previous CCV fails acceptance criteria.

10.9.4 Acceptance Criteria for Continuing Calibration Verification (CCV)

10.9.4.1 Detected Analytes (≥ RL)

For any analyte detected at or above the reporting limit (RL) in client samples, the percent difference (%D) for that analyte in the preceding and following CCVs (i.e., bracketing CCVs) or 12-hour calibration, on the column used for quantitation, must be within ± 15% and 10% for EPA 614. Method 8141B allows for ± 20% but does not allow the use of the grand mean that is explained in section 10.6.4.2 and Table 5.

Note: Anilazine and Naled are known poor performers and are allowed a %D of ± 30%.

In some cases, the nature of the samples being analyzed may be the cause of a failing %D. When the %D for an analyte falls outside acceptance limits in the CCV, and that analyte is detected in any or all of the associated samples, then those samples must be reanalyzed to prove a matrix effect. If the drift is repeated in the reanalysis, the analyst must generate an NCM for this occurrence to explain that the drift was most likely attributable to the sample matrix and that the samples may be diluted and reanalyzed to minimize the effect if so desired by the client.

Refer to Section 12 for which result to report.

The %D is calculated as follows:

$$\%D = \frac{\text{Measured Conc} - \text{Theoretical Conc}}{\text{Theoretical Conc}} \times 100$$ *Equation 10*

10.9.4.2 Analytes Not Detected (< RL)

For any analyte not detected in client samples, the %D for that analyte in the bracketing CCVs should also be within ± 15% (8141A), ± 10% (614) and ± 20% (8141B). However, the analysis is acceptable for Method 8141A if the average of the %D values for all the analytes is within ± 15% and the %D for any individual analyte is within ± 30%. The average %D is calculated by summing all the %D results in the calibration and dividing by the number of analytes. If an average %D is used and the %D for any individual analytes falls outside of ± 30%, then additional evaluation is needed as summarized in Table 5.

10.10 Retention Time Windows

10.10.1 Retention time windows must be determined for all analytes.

10.10.1.1 Determine new RT windows each time a new column is installed, when RT drift indicates that a new window needs to be established, or annually, whichever is most frequent.

10.10.1.2 Make an injection of all analytes of interest each day over a three-day period.

10.10.1.3 Calculate the mean retention time and associated standard deviation of the three retention times for each analyte, as follows:

$$\text{Mean RT} = \overline{RT} = \frac{\sum_{i=1}^{n} RT_i}{n} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n} \left(RT_i - \overline{RT}\right)^2}{n-1}}$$ *Equations 11 & 12*

Where:

RT_i = Retention time for the i^{th} injection.

n = Number of injections (typically 3).

SD = Standard deviation.

10.10.1.4 Set the width of the RT window for each analyte at ± 3 standard deviations of the mean RT for that analyte.

10.10.1.5 The center of the RT window for an analyte is the RT for that analyte from the last of the three standards measured for the 72-hour study.

10.10.2 The center of the window for each analyte is updated with the RT from the level 5 standard of the ICAL, or the CCV, at the beginning of the analytical sequence. The width of each window remains the same until new windows are generated following the installation of a new column, or in response to an RT failure. The RT window width may be expanded if the RT drift observed in the ICAL is greater than

the established window. The expanded window is noted on the ICAL checklist.

10.10.3 If the RT window as calculated above is less than ± 0.03 minute, use ± 0.03 minute as the RT window. This allows for slight variations in retention times caused by sample matrix.

10.10.4 The laboratory must calculate new retention time windows each time a new column is installed. The new windows must be generated within one week of the installation of the new column. Until these standards have been run on the new column, the retention time windows from the old column may be used, updated with the retention times from the new initial calibration.

10.10.5 The analyst must review peak assignments in standards, at least annually, any time retention time windows are recalculated or when stock standards are replaced. Sample chromatograms are provided in the attachment for verification of elution order on the columns specified in Section 6.1.2.

11.0 Procedure

11.1 One-time procedural variations are allowed only if deemed necessary in the professional judgment of supervision to accommodate variation in sample matrix, radioactivity, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using an NCM. The NCM is automatically sent to the laboratory Project Manager by e-mail so that the client can be notified as appropriate. The QA group periodically reviews NCMs for potential trends. The NCM process is described in more detail in SOP # DV-QA-0031. The NCM shall be filed in the project file and addressed in the case narrative.

11.2 Any deviations from this procedure identified after the work has been completed must be documented in an NCM, with a cause and corrective action described.

11.3 Sample Extraction

Instructions for extracting aqueous samples are detailed in SOP DV-OP-0006. Instructions for extracting solid samples are detailed in SOP DV-OP-0010. Instructions for concentrating extracts are detailed in SOP DV-OP-0007.

11.4 Gas Chromatography

Chromatographic conditions for this method are presented in Table 2. Use the ChemStation interface to establish instrument operating conditions for the GC. Raw data obtained by the ChemStation software is transferred to the TARGET DB database for further processing. The data analysis method, including peak processing and integration parameters, calibration, RT windows, and compound identification parameters, is set up in the TARGET DB software.

Instrument Maintenance

Before the start of any daily sequence the instrument system should be evaluated for possible maintenance. If the previous run ended with a failing continuing calibration then the system should be maintained to bring it back into control. The injector septum should be changed after about 200 injections have been completed. If the last CCV that was analyzed indicated a high response then a simple liner change is typically sufficient to bring the system back into control. Analysis of a few solvent blanks or a system bake out may be necessary to

drive out any residual contamination on the column. A reduced response may indicate that the system needs to be evaluated for leaks. Poor peak shape may necessitate clipping a loop out of the analytical column. If this fails to solve the peak shape problem then replacement of the columns may be indicated. The goal is to maintain the system as close to top condition as possible as was observed when new columns and injector parts were installed. Re-calibration should not be used to correct for maintenance related issues. Always document any maintenance procedure in the maintenance logbook.

Bead Lighting Procedure

In order to protect the bead from damage and maximize bead lifetime a specific bead lighting procedure must be followed. Begin by turning the hydrogen pressure in the detector to 3.0 (do not exceed 4). Turn the bead voltage to 2.5 (do not exceed 4 at any time during the lighting process or use). Increase the voltage in increments of 0.1 units until the voltage reaches 3.0. If the bead indicates lighting (as evidenced by a sudden and significant increase in signal) at less than 3.0, for example when the bead is new, then slow the voltage increase increment to 0.02 prior to the anticipated voltage at which lighting should occur. If lighting has not occurred by the time a voltage of 3.0 is reached then reduce the voltage increase increment to 0.02 (after you reach 3.0) until the bead lights. After a sustained bead light up is obtained then increase the voltage an additional 0.01 units in order to insure that the bead stays lit. Increase the hydrogen at this time until the output signal reads a signal of 10.

11.5 Sample Introduction

11.5.1 Analytes are introduced by direct injection of the extract. Samples, standards, and QC must be introduced using the same procedure.

11.5.2 Allow all extracts and standards to warm to room temperature before injection.

11.5.3 To prepare a sample for injection, transfer 250 µL of the sample extract to a conical vial. Then add 5 µL of the internal standard solution.

11.5.4 Cap the vial and invert it approximately 10 times to ensure a uniform mixture.

11.5.5 Use the ChemStation interface to set up and run the analytical sequence. Sample injection and analysis are automated and may proceed unattended.

11.6 Analytical Sequence

An analytical sequence starts with an initial calibration (Section 10.6) or a daily calibration verification standard and ends with a daily calibration verification standard.

11.6.1 The daily calibration includes analysis of the 12-hour calibration sequence (i.e., a mid-level calibration standard (CCV) and an instrument blank as described in Section 10.9.1) and updating the retention time windows (Section 10.10.2).

11.6.2 The CCV is analyzed after every 20 samples, including matrix spikes, LCSs and method blanks. Some programs require a CCV after every 10 samples. The CCV must be analyzed every 12 hours to maintain the 12-hour calibration sequence or at the end of the sequence if no further analyses are performed.

11.6.3 If there is a break in the analytical sequence of greater than 12 hours, a new analytical sequence must be started with a daily calibration. Any samples that were not bracketed by calibration verification standards must be reinjected with the appropriate bracketing standards.

11.7 Daily Retention Time Windows

The centers of the retention time (RT) windows determined in Section 10.10 are adjusted to the RT of each analyte as determined in the 12-hour calibration verification. The centers of the RT windows must be updated at the beginning of each analytical sequence, but not for any other calibration verification standards.

11.8 Upon completion of the analytical sequence, transfer the raw chromatography data to the TARGET DB database for further processing.

11.8.1 Review chromatograms online and determine whether manual data integrations are necessary.

11.8.2 All manual integrations must be justified and documented. See DV-QA-011P for requirements for manual integration.

11.8.3 Manual integrations may be processed using an automated macro, which prints the before and after chromatograms and the reason for the change, and attaches the analyst's electronic signature.

11.8.4 Alternatively, the manual integration may be processed manually. In the latter case, print both the both the before and after chromatograms and record the reason for the change and initial and date the after chromatogram.

11.8.5 Before and after chromatograms must be of sufficient scale to allow an independent reviewer to evaluate the manual integration.

11.9 Compile the raw data for all the samples and QC samples in a batch. The analytical batch is defined as containing no more than 20 samples, which include field samples and the MS and MSD.

11.9.1 Transfer the data to the LIMS system.

11.9.2 Perform a level 1 data review and document the review on the data review checklist, GC Data Review Checklist/Batch Summary. (See SOP DV-QA-0020.)

11.9.3 Submit the data package and review checklist for the level 2 review. The data review process is explained in SOP DV-QA-0020. Review of all manual integrations must be documented and the level 2 review is documented on the review checklist initiated at the level 1 review.

12.0 Calculations / Data Reduction

12.1 Qualitative Identification

12.1.1 Tentative identification occurs when a peak is found on the primary column within the retention time window for an analyte, at a concentration above the reporting

limit, or above the MDL if J flags are required. Identification is confirmed if a peak is also present in the retention time window for that analyte on the confirmation column, at a concentration greater than the reporting limit (MDL if J flag confirmation required).

12.1.2 The experience of the analyst should weigh heavily in the interpretation of the chromatogram. For example, sample matrix or laboratory temperature fluctuation may result in variation of retention times. If a RT shift greater than the RT window occurs for a reported compound then the situation must be explained in an NCM.

12.2 Dual-Column Quantitation and Reporting

12.2.1 Each sample is analyzed on two different columns at the same time. The laboratory designates a primary column based on optimal separation of the compounds of interest and other desirable chromatographic characteristics. The result from the primary column is normally reported. The result from the secondary (confirmation) column is reported if any of the following is true:

12.2.1.1 There is obvious chromatographic interference on the primary column.

12.2.1.2 The difference between the result for the primary column and the result for the secondary column is > 40% and chromatographic interference is evident on the primary column.

12.2.1.3 The continuing calibration verification, bracketing standard, or surrogate recovery fails on the primary column, but is acceptable on the secondary column. However, if the difference between the primary column result and the secondary column result is > 40% and the primary column calibration fails, then the sample must be evaluated for reanalysis.

12.2.2 Percent Difference Calculation

The RPD between two results is calculated using the following equation:

$$\%RPD = \frac{\left|R_1 - R_2\right|}{\frac{1}{2}\left(R_1 + R_2\right)} \times 100\% \qquad \textit{Equation 13}$$

Where R_1 is the result for the first column and R_2 is the result for the second column.

12.2.3 Dual Column Results With > 40% RPD

12.2.3.1 If the relative percent difference (RPD) between the responses on the two columns is greater than 40%, the higher of the two results is reported unless there is obvious interference documented on the chromatogram.

12.2.3.2 If there is visible positive interference, e.g., co-eluting peaks, elevated baseline, etc., for one column and not the other, then report the results from the column without the interference with the appropriate data qualifier flag, footnote, and/or narrative comment in the final report.

12.2.3.3 If there is visible positive interference for both columns, then report the lower of the two results with the appropriate flag, footnote, and/or narrative comment in the final report.

12.3 Surrogate Recovery

12.3.1 Concentrations of surrogate compounds are calculated using the same equations as for the target compounds. The response factor from the initial calibration is used. Surrogate recovery is calculated as follows:

$$\%\text{Recovery} = \frac{\text{Measured Conc}}{\text{Theoretical Conc}} \times 100 \qquad \textit{Equation 14}$$

12.4 Calibration Range and Sample Dilutions

If the concentration of any analyte exceeds the working range as defined by the calibration standards, then the sample must be diluted with hexane (record the hexane lot number in the run sequence) and reanalyzed. Dilutions should target the most concentrated analyte in the upper half (over 50% of the high level standard) of the calibration range. Samples that were analyzed immediately following the high sample must be evaluated for carryover. If the samples have results at or above the RL for the analyte(s) that were found to be over the calibration range in the high sample, they must be reanalyzed to rule out carryover. It may also be necessary to dilute samples because of matrix interferences.

12.4.1 If the initial diluted run has no hits or hits below 20% if the calibration range, and the matrix allows for analysis at a lesser dilution, then the sample must be reanalyzed at a dilution targeted to bring the largest hit above 50% of the calibration range.

12.4.2 Guidance for Dilutions Due to Matrix Interference

If the sample is initially run at a dilution and only minor matrix peaks are present, then the sample should be reanalyzed at a more concentrated dilution. Analyst judgment is required to determine the most concentrated dilution that will not result in instrument contamination. Ideally, the dilution chosen will make the response of the matrix interferences equal to approximately half the response of the mid-level calibration standard.

12.4.3 Reporting Dilutions

Some programs (e.g., South Carolina and AFCEE) and some projects require reporting of multiple dilutions (check special requirements in LIMS). In other cases, the most concentrated dilution with no target compounds above the calibration range will be reported.

12.5 Interferences Observed in Samples

12.5.1 Dual column analysis does not entirely eliminate interfering compounds. Complex samples with high background levels of interfering organic compounds can

produce false positive and/or false negative results. The analyst must use appropriate judgment to take action as the situation warrants.

12.5.2 Suspected Negative Interferences

If peak detection is prevented by interferences, further cleanup should be attempted (see SOP DV-OP-0007). Elevation of reporting levels and/or lack of positive identification must be addressed in the case narrative.

12.5.3 Suspected Positive Interferences

If no further cleanup is reasonable and interferences are evident that are suspected of causing false positive results, consult with the laboratory Project Manager to determine if analysis using additional confirmation techniques is appropriate for the project. Use of additional confirmation columns is another possible option. At a minimum, the Data Review Template prepared by the analyst should include the following comment for inclusion in the case narrative:

"Based on review of the chromatograms for samples _____, it is my opinion that the evident interferences may be causing false results.

Date _____ Analyst _____"

12.6 Calculation of Sample Results

12.6.1 Depending on the calibration function used, the concentration of the analyte in the sample extract is calculated as follows (see Section 10.7 for details on establishing the calibration function):

Average Response Factor: $$C_e = \frac{A_s C_{is}}{A_{is} \overline{RF}}$$ *Equation 16*

Linear Regression: $$C_e = \frac{\left[\frac{A_s C_{is}}{A_{is}} - b\right]}{a}$$ *Equation 17*

Non-linear Regression: $$C_e = f\left(\frac{A_s C_{is}}{A_{is}}\right)$$ *Equation 18*

Where:

- C_e = Concentration of the analyte in the sample extract (µg/mL).
- A_s = Peak area for the analyte in the sample extract injection.
- C_{is} = Concentration of the internal standard in the sample extract (µg/mL).
- A_{is} = Peak area for the internal standard in the sample extract.
- b = y-intercept of the calibration fit.
- a = Slope of the calibration fit.
- $f( )$ = Mathematical function established by the non-linear regression.

12.7 The concentrations of target analytes in the original aqueous sample are calculated as follows:

$$C_S = \frac{C_e V_e}{V_S} \times DF \qquad \textit{Equation 19}$$

Where:

C_S = Concentration of target analyte in the original sample, μg/L.
C_e = Concentration of the target analyte in the sample extract as determined by the calibration function, μg/mL.
V_e = The final volume of the sample extract, mL.
V_s = The volume of the original sample that was extracted, L.
DF = Dilution factor, if applicable.

12.8 The concentrations of target analytes in the original solid sample are calculated as follows

$$C_S = \frac{C_e V_e}{W_S} \times DF \qquad \textit{Equation 20}$$

Where:

C_S = Concentration of target analyte in the original sample, μg/kg.
C_e = Concentration of the target analyte in the sample extract as determined by the calibration function, μg/mL.
V_e = The final volume of the sample extract, mL.
W_s = The weight of the original sample that was extracted, kg.
DF = Dilution factor, if applicable.

12.9 LCS and Surrogate Spike Recovery Calculation

LCS and surrogate spike recoveries are calculated using the following equation:

$$\%\text{Recovery} = \frac{\text{Concentration (or amount) found}}{\text{Concentration (or amount) spiked}} \times 100\% \qquad \textit{Equation 21}$$

12.10 MS and MSD Recovery Calculation

Matrix spike recoveries are calculated as follows:

$$\text{MS or MSD } \%\text{Recovery} = \left(\frac{SSR - SR}{SA} \right) \times 100\% \qquad \textit{Equation 22}$$

Where:

SSR = Measured concentration in spiked sample.
SR = Measured concentration in unspiked sample.
SA = Concentration of spike added to sample.

12.11 MS/MSD RPD Calculation

The relative percent difference between the MS and MSD is calculated as follows:

$$\%RPD = \frac{\left|R_1 - R_2\right|}{\frac{1}{2}\left(R_1 + R_2\right)} \times 100\% \qquad \textit{Equation 23}$$

Where R_1 is the result for the MS and R_2 is the result for the MSD.

12.12 A second-level technical review of the organic data is performed prior to data reporting. This review is performed by a peer or supervisor using the guidelines and checklists detailed in SOP DV-QA-0020.

13.0 Method Performance

13.1 Initial Demonstration of Capability

13.1.1 An initial demonstration of capability for each method must be performed prior to analyzing samples.

13.1.2 For the standard analyte list, the initial demonstration consists of the preparation and analysis of a QC check sample containing all of the standard analytes for the method, as well as a method detection limit (MDL) study (described in Section 13.2 below).

13.1.3 Four aliquots of the QC check sample (independent source from the calibration) are analyzed with the same procedures used to analyze samples, including sample preparation.

13.1.4 The mean recovery and standard deviation are calculated for each analyte of interest. These results are compared with the established or project-specific acceptance criteria. All four results must meet acceptance criteria before the method can be used to analyze samples.

13.1.5 For non-standard analytes, an MDL study must be performed and calibration curve generated before analyzing any samples, unless lesser requirements are previously agreed to with the client. In any event, the minimum initial demonstration required is successful analysis of an extracted standard at the reporting limit and a single point calibration.

13.2 Method Detection Limit (MDL)

An initial method detection limit study is performed for each analyte and each sample matrix type in accordance with Policy DV-QA-005P. An MDL verification is performed once a year to satisfy state certification requirements. For DoD, AFCEE, and DOE projects, an MDL verification is performed quarterly. MDLs are stored in LIMS.

13.3 Analyst Training and Qualification

13.3.1 The group leader is responsible for ensuring that this procedure is performed by an associate who has been properly trained in its use and has the required

experience. See requirements for demonstration of analyst proficiency in SOP DV-QA-0024.

13.3.2 Each analyst performing the method must complete a demonstration of capability (DOC) by successfully preparing and/or analyzing four consecutive LCSs, or a blind performance evaluation (PE) sample, or other acceptable QC samples. The results of the DOC study are summarized in the NELAC format, as described in SOP DV-QA-0024. DOCs are approved by the Quality Assurance Manager and the Technical Director. DOC records are maintained by the QA staff in the central training files. Analysts who continue to perform the method must successfully complete a demonstration of capability annually.

14.0 Pollution Control

Standards and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents requiring disposal.

15.0 Waste Management

15.1 All waste will be disposed of in accordance with Federal, State, and local regulations. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this procedure, the policies in Section 13, "Waste Management and Pollution Prevention", of the Environmental Health and Safety Manual, and DV-HS-001P, "Waste Management Program."

15.2 The following waste streams are produced when this method is carried out:

15.2.1 Vials containing sample extracts: Expired Extract Vials – Waste Stream A

15.2.2 Expired reagents and standards – Contact Waste Coordinator

NOTE: Radioactive and potentially radioactive waste must be segregated from non-radioactive waste as appropriate. Contact the Radioactive Waste Coordinator for proper management of radioactive or potentially radioactive waste generated by this procedure.

16.0 References

16.1 SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, Third Edition and all promulgated updates, EPA Office of Solid Waste, January 2005.

16.1.1 Method 8141A, Organophosphorous Compounds by Gas Chromatography: Capillary Column Technique, Revision 1, September 1994.

16.1.2 Method 8141B, Organophosphorus Compounds by Gas Chromatography. Final Update IV, Revision 2, February 2007, Method 8141B.

16.1.3 Method 8000B, Determinative Chromatographic Separations, Revision 2, December 1996.

16.1.4 Method 8000C, Determinative Chromatographic Separations, Revision 3, March 2003.

16.1.5 Method 3510C, Separatory Funnel Liquid-Liquid Extraction, Revision 3, December 1996.

16.1.6 Method 3540C, Soxhlet Extraction, Revision 3, December 1996.

16.2 Title 40, Code of Federal Regulations (40CFR), Part 136 – Guidelines Establishing Test Procedures for the Analysis of Pollutants, Appendix A, -- Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, Method 614, Organophosphorus Pesticides.

17.0 Method Modifications:

Item	Method No.	Modification
1	614	Method 614 specifies the use of packed columns, or any other columns that provide equivalent performance. The laboratory uses capillary columns for this analysis, which give superior performance.
2	614	This SOP requires a minimum of five calibration levels, whereas Method 614 requires at least three calibration concentrations.
3	614	Method 614 requires an RSD of <10% or the use of a calibration curve, with no stated linearity requirement. This SOP includes a linearity acceptance limit for a weighted or unweighted linear regression, $r \geq 0.990$.
4	614	Method 614 does not include any requirements for the agreement for dual-column results. This SOP requires that the difference must < 40%, or the result is qualified.
5	614	This SOP includes details for performing retention time studies. There are none in the source method.
6	8000B 8000C	These methods allow the use of third-order calibration curves. TestAmerica Denver does not allow third-order curves.
7	8141A 8141B 614	For calibration verification, Anilazine and Naled are documented poor performers and are allowed a %D of ± 50%.

18.0 Attachments

Table 1. Standard Analyte List and Reporting Limits
Table 2. Suggested Instrument Conditions
Table 3. Calibration Concentrations and Recipes
Table 4. LCS, MS, and MSD Spike Compounds
Table 5. Evaluation Criteria and Corrective Actions for Continuing Calibration Verification
Attachment 1 Example Standard Chromatogram – Column RTx-1MS – Full chromatogram
Attachment 2 Example Standard Chromatogram – Column RTx-1MS – Expanded Views

Attachment 3 Example Standard Chromatogram – Column RTx-OPPest – Full chromatogram

Attachment 4 Example Standard Chromatogram – Column RTx- OPPest – Expanded Views

Attachment 5 Example Standard Chromatogram – Column RTx- OPPest 2 – Full chromatogram

Attachment 6 Example Standard Chromatogram – Column RTx- OPPest 2 – Expanded Views

19.0 Revision History

- Revision 8.0, dated 15 July 2013
  - Added use of second ICV solution in Sections 7.6, 10.8 and Table 3.
  - Updated Sections 9.1 and 11.1 to reflect current practice.
- Revision 7.0, dated 04 January 2013
  - Added section 9.9 for 2012 MUR QC requirements
  - Updated 10.9.4.1 and 10.9.4.2 to include EPA 614 CCV criteria.
- Revision 6.1, dated 16 May 2012
  - Annual Technical Review.
  - Revised Section 10.10.1.1 regarding frequency of new RT windows
  - Inserted paragraphs on instrument maintenance and bead lighting in Section 11.4
  - Formatting and grammatical corrections throughout SOP
- Revision 6, dated 16 May 2011
  - Added a Supelco standard to 7.1.6.
  - Acetone used as diluent in section 7.3.1.
  - Added standard preparation detail to 7.7 and 7.8.
  - Changed allowable MB concentration to ½ the RL and added detail to corrective action in section 9.4.
  - Added other acceptance criteria to section 9.8 and dilution details to the corrective action.
  - Specified that 8141A has +/-15% criteria in section 10.5.
  - Revised section 10.6.2 to include the method 8000 recommendation for two CCV levels.
  - Clarified that method 8141B does not allow the use of a grand mean for CCV in section 10.6.4.1.
  - Added a line indicating data transfer to LIMS to section 11.9.
  - Included a note in section 12.1.2 that RT shifts must be narrated.
  - Included a note to section 12.4 to use hexane and record the lot number.
  - Updated the RLs in table 1.
  - Updated the instrument conditions in table 2.
  - Updated the standard prep for L7 in table 3.
  - Source methods review
- Revision 5, dated 15 May 2010
  - Edited section 10.5 ICV criteria to include poor performers.
- Revision 4, dated 26 August 2009

  - Updated Standard list in Section 7.1.6
  - Changed Table 3 to current concentrations and added recipes.

- Revision 3, dated 16 October 2008
  - Updated to include 8141B requirements
- Revision 2, dated 22 October 2007
  - Integration for TestAmerica and STL operations.
  - Updated concentrations of certified stock standards in section 7.1.6
  - Updated the continuing calibration verification level from 4 to 5 in sections 10.6.2 and 10.7.2.
  - Updated analyte names in Tables 1, 3, and 4 for consistency.
  - Updated the Initial Temp for the suggested Instrumental Conditions Table from 100°C to 110°C.
  - Corrected minor typographical errors.
- Revision 1, dated 27 October 2006
  - Reformatted SOP to comply with current STL Denver guidance (Policy QA-001).
  - Revised Sections 5 (Safety), 14 (Pollution Prevention), and 16 (Waste Management) to comply with STL Corporate requirements.
  - Updated spike solution information in Section 7 and Table 4.
  - Revised Section 10 and added Table 5 to include more detailed evaluation criteria for continuing calibration verification.
- Revision 0, dated 8 October 2001
  - Initial release

Table 1.

Standard Analyte List and Reporting Limits

Compound	CAS Number	Reporting Limits	
		Water (μg/L)	Solid (μg/kg)
Azinphos methyl	86-50-0	2.5	13
Bolstar (Suprofos)	35400-43-2	1.0	13
Chlorpyrifos	2921-88-2	1.5	20
Coumaphos	56-72-4	1.0	13
Demeton, O and S	8065-48-3	3.0	39
Diazinon	333-41-5	0.5	22
Dichlorvos	62-73-7	0.5	23
Disulfoton	298-04-4	1.0	48
EPN	2104-64-5	1.2	13
Ethoprop	13194-48-4	1.5	15
Fensulfothion	115-90-2	2.5	25
Fenthion	55-38-9	2.5	33
Malathion	121-75-5	2.0	15
Merphos	150-50-5	5.0	30
Methyl Parathion	298-00-0	4.0	20
Mevinphos	7786-34-7	6.2	15
Naled	300-76-5	2.0	70
Phorate	298-02-2	1.2	20
Ronnel	299-84-3	10	46
Tetrachlorvinphos (Stirophos)	22248-79-9	3.5	15
Tokuthion	34643-46-4	1.6	20
Trichloronate	327-98-0	1.5	20
O,O',O"-Triethyl phosphorothioate	126-68-1	0.5	39
Thionazin	297-97-2	1.0	18
Sulfotepp	3689-24-5	1.5	20
Dimethoate	60-51-5	1.5	22
Ethyl Parathion (Parathion)	56-38-2	1.0	18
Famphur	52-85-7	1.0	13
Additional Compounds			
Anilazine	101-05-3	10	40
Atrazine	1912-24-9	10	67
Propazine	139-40-2	10	67
Simazine	122-34-9	10	67
Chlorpyrifos-methyl	5598-13-0	0.5	--
Azinphos ethyl	2642-71-9	0.7	33
Carbophenthion	786-19-6	1.0	33
Methyl carbophenthion	953-17-3	0.8	33
Phosmet	732-11-6	1.5	67

Table 2.

Suggested Instrumental Conditions

Parameter	Recommended Conditions[1]
Injection Port Temp	250 °C
Detector Temp	325 °C
Initial Temp	80 °C (D) 75 °C (D2) then hold for 0.5 min
Temperature Program	(**ramp A**) 6°C/minute (D) 11°C/minute (D2) (**ramp B**) 12°C/minute (D and D2) (**ramp C**) 4°C/minute (D) 8°C/minute (D2) **(ramp D**) 2°C/minute (D) 30°C/minute (D2) (**ramp E**) 30°C/minute (D only)
Final Temp	(**A**) 160°C (D and D2) (**B**) 180°C (D) 185°C (D2) (**C**) 202°C (D) 210°C (D2) (**D**) 210°C (D) 305°C (D2) (**E**) 305° (D)
Final Hold Time	*(**A**) 0.00 minute (D and D2)* *(**B**) 0 minute (D) 1 minute (D2)* *(**C**) 0 minutes (D and D2)* *(**D**) 0 minute (D) 2 minutes (D2)* *(**E**) 2 minute (D)*
Column 1	Rtx®-OPP, 30 meter X 0.32 mm X 0.5 μm film
Column 2	Rtx®-1MS, 30 meter X 0.32 mm X 0.25μm film
Injection Volume	2 μL
Carrier and Detector Gases	Helium and Hydrogen
Make-up Gas	Nitrogen

[1] D and D2 are Instrument IDs.

Table 3.

Calibration Concentrations and Recipes

Calibration Level	Conc.	Final Volume	Recipe
ICAL Level 7	5 μg/mL	10 mL	0.25 mL Restek 8140/8141 OP Pesticides Cal Mix A
			0.25 mL Restek Custom 8141 Standard
			0.5 mL ChemService Carbophenothion Methyl
			0.5 mL Accustandard Chlormefos (surr.)
			0.5 mL Accustandard Triphenyl Phosphate (surr.)
ICAL Level 6	4 μg/mL	0.250 mL	0.200 mL of ICAL Level 7
ICAL Level 5	3 μg/mL	0.250 mL	0.150 mL of ICAL Level 7
ICAL Level 4	2 μg/mL	0.250 mL	0.100 mL of ICAL Level 7
ICAL Level 3	1 μg/mL	0.250 mL	0.050 mL of ICAL Level 7
ICAL Level 2	0.5 μg/mL	0.250 mL	0.025 mL of ICAL Level 7
ICAL Level 1	0.2 μg/mL	0.250 mL	0.010 mL of ICAL Level 7
Second Source 1	2 μg/mL	5 mL	0.050 mL Accustandard 8140 Cal Mix A
			0.050 mL Accustandard Custom 8141 Standard
			0.100 mL Accustandard Methyl Trithion
			0.010 mL Accustandard Chlormefos (surr.)
			0.010 mL Accustandard Triphenyl Phosphate (surr.)
Second Source 2	5 μg/mL	5 mL	0.125 mL Supelco 8140 Stock
			0.250 mL Accustandard Methyl Trithion
Continuing Calibration (CCV)	2.5 μg/mL	10 mL	5.0 mL of ICAL Level 7

* All standards are brought to the final volume in Hexane:Acetone (90:10)
* Actual concentrations of each component in each standard is stored in the Reagent Module in the LIMS

Table 4.

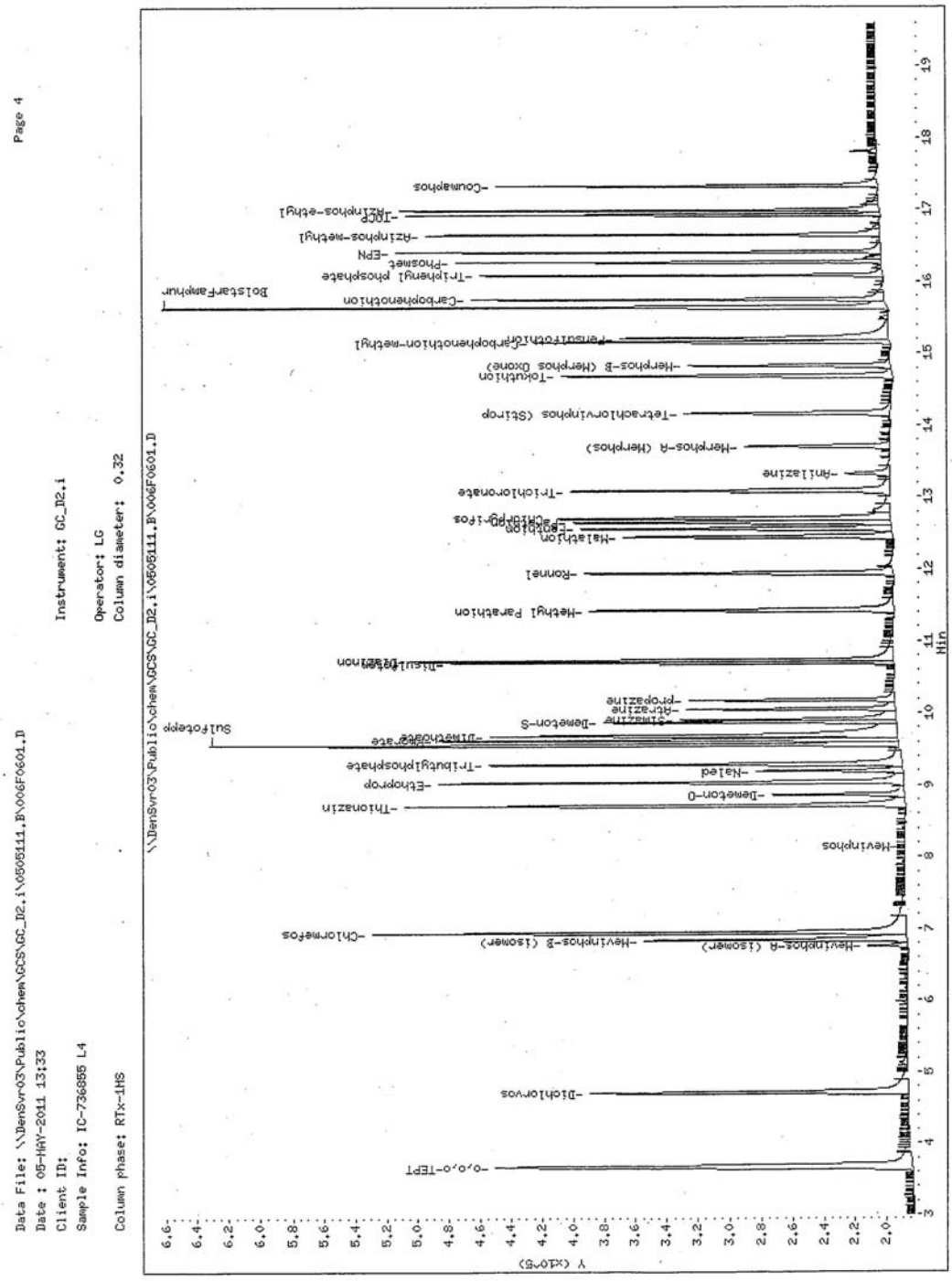
LCS, MS, and MSD Spike Compounds

Spike Solution:			
Compound	**Concentration (μg/mL)**	**SW846 8141A Control Compound**	**EPA Method 614 Control Compound**
O, O', O"-Triethylphosphorothioate	4.0	X	
Thionazin	4.0	X	
Famphur	4.0	X	
Anilazine	4.0		
Atrazine	4.0	X	
Propazine	4.0		
Simazine	4.0	X	
Fenthion	4.0	X	
Merphos	4.0		
Methyl parathion	4.0	X	X
Mevinphos	4.0	X	
Naled	4.0		
Phorate	4.0	X	X
Ronnel	4.0	X	
Tetrachlorvinphos (Stirophos)	4.0	X	
Tokuthion	4.0		
Trichloronate	4.0	X	
Azinphos-methyl	4.0	X	
Bolstar	4.0		
Chlorpyrifos	4.0	X	
Coumaphos	4.0	X	
Demeton	4.0	X	X
Diazinon	4.0	X	X
Dichlorvos	4.0	X	
Disulfoton	4.0	X	
Ethoprop	4.0	X	
Fensulfothion	4.0	X	
Dimethoate	4.0	X	
EPN	4.0		
Ethyl parathion (Parathion)	4.0	X	
Malathion	4.0	X	X
Sulfotep	4.0	X	
Surrogate Solution:			
Chlormephos	2.0	X	X
Triphenylphosphate	2.0	X	X

Table 5.
Evaluation Criteria and Corrective Actions for Continuing Calibration Verification

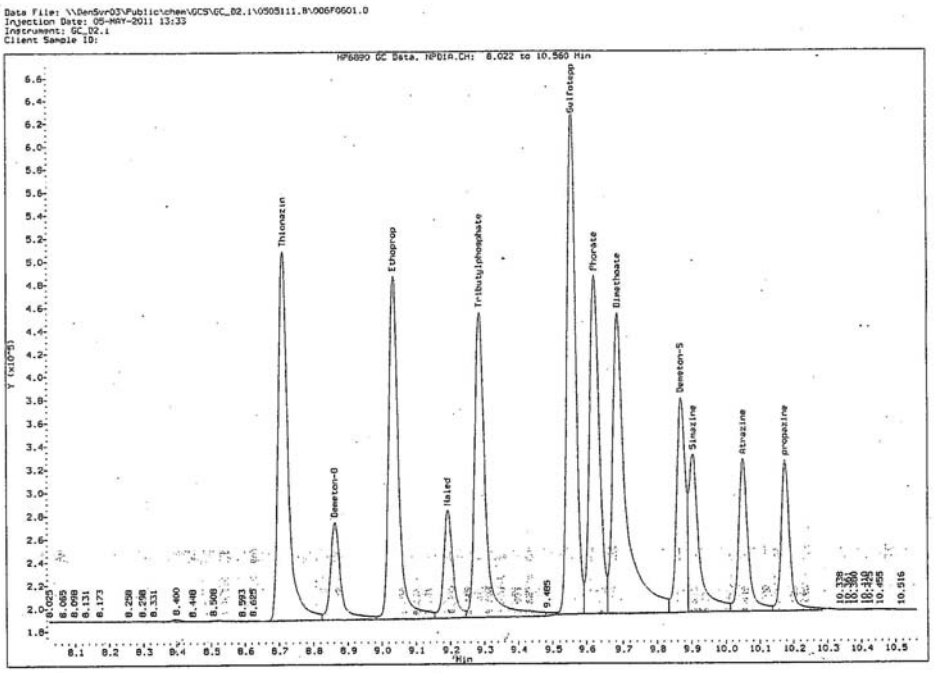
Evaluation Criteria for a Specific Analyte				Evaluation / Corrective Actions
Average %D	**Individual %D**	**RL Standard**	**Client Samples**	
N/A	± 15% ± 20% (8141B)	N/A	≥ RL	Calibration is verified for the analyte(s) detected in the sample; no action required.
N/A	Outside of ± 15% ± 20% (8141B)	N/A	≥ RL	Calibration is not verified for the analyte(s) detected in the sample. The sample must be re-analyzed using a verified calibration.
± 15%	± 30%	N/A	ND	Calibration is acceptable because analytes were not detected in the sample. An NCM is required.
Outside of ± 15%	N/A	N/A	N/A	Calibration is <u>not</u> verified and corrective action must be taken. **NOTE**: The exception to this may be those cases where the client has requested a small subset of the analytes typically measured by the method and the %D for each of those analytes is within ± 15%. Corrective action may include clipping the column, changing the liner, or other minor instrument adjustments, followed by reanalyzing the standard twice. If both results pass acceptance criteria, the calibration may be used to process samples. If the overall average %D still varies by more than ±15%, a new calibration curve must be prepared. Reanalyze any samples that were either preceded by or followed by the failed CCV using a verified calibration.
± 15%	< -30% (low)	Detected	ND	Sample results are acceptable because the RL standard indicates that the analyte would have been detected if present in the sample. Explain in an NCM. (**note:** If results are required to be reported to the MDL, client approval is required.)
± 15%	< -30% (low)	ND	ND	Analyte was not detected in the RL standard, possibly as the result of a calibration drift in the negative direction, and therefore one cannot be sure that the analyte would have been detected in the sample if present. Reanalyze samples with verified calibration. In the event that re-analysis substantiates that sample matrix is causing the drift, notify PM and discuss analytical approach with client.
± 15%	> +30% (high)	N/A	ND	Sample results are acceptable because the CCV failed high and the analyte was detected in the RL standard, so if the analyte were present in the sample, it would definitely have been detected. Explain in an NCM.

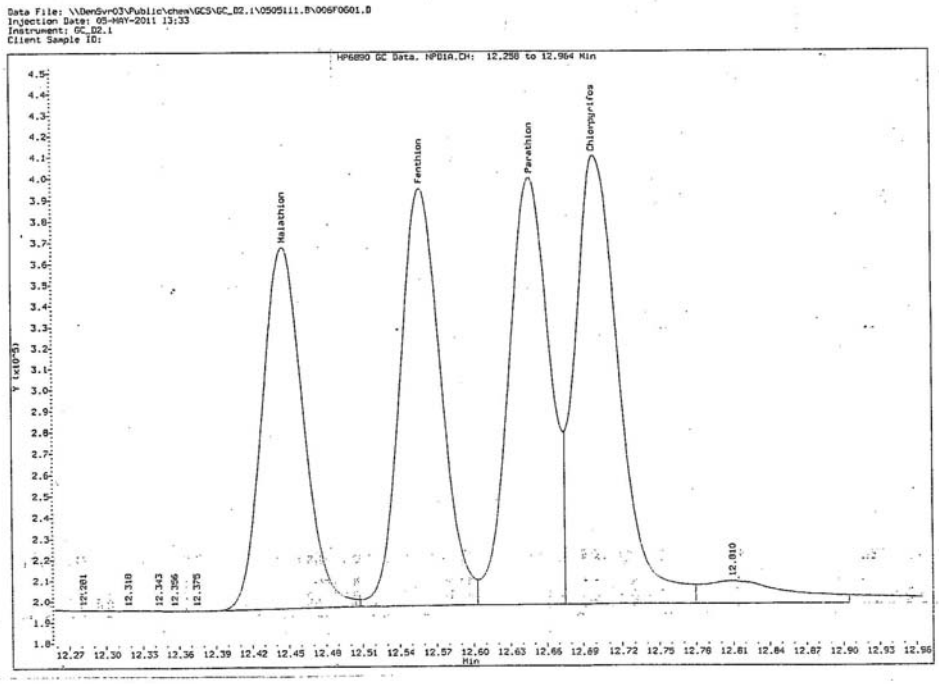
Attachment 1
Example Standard Chromatogram –Column: RTx-1MS
Full Chromatogram



Note: Absolute retention times may vary due to actual column length and condition.

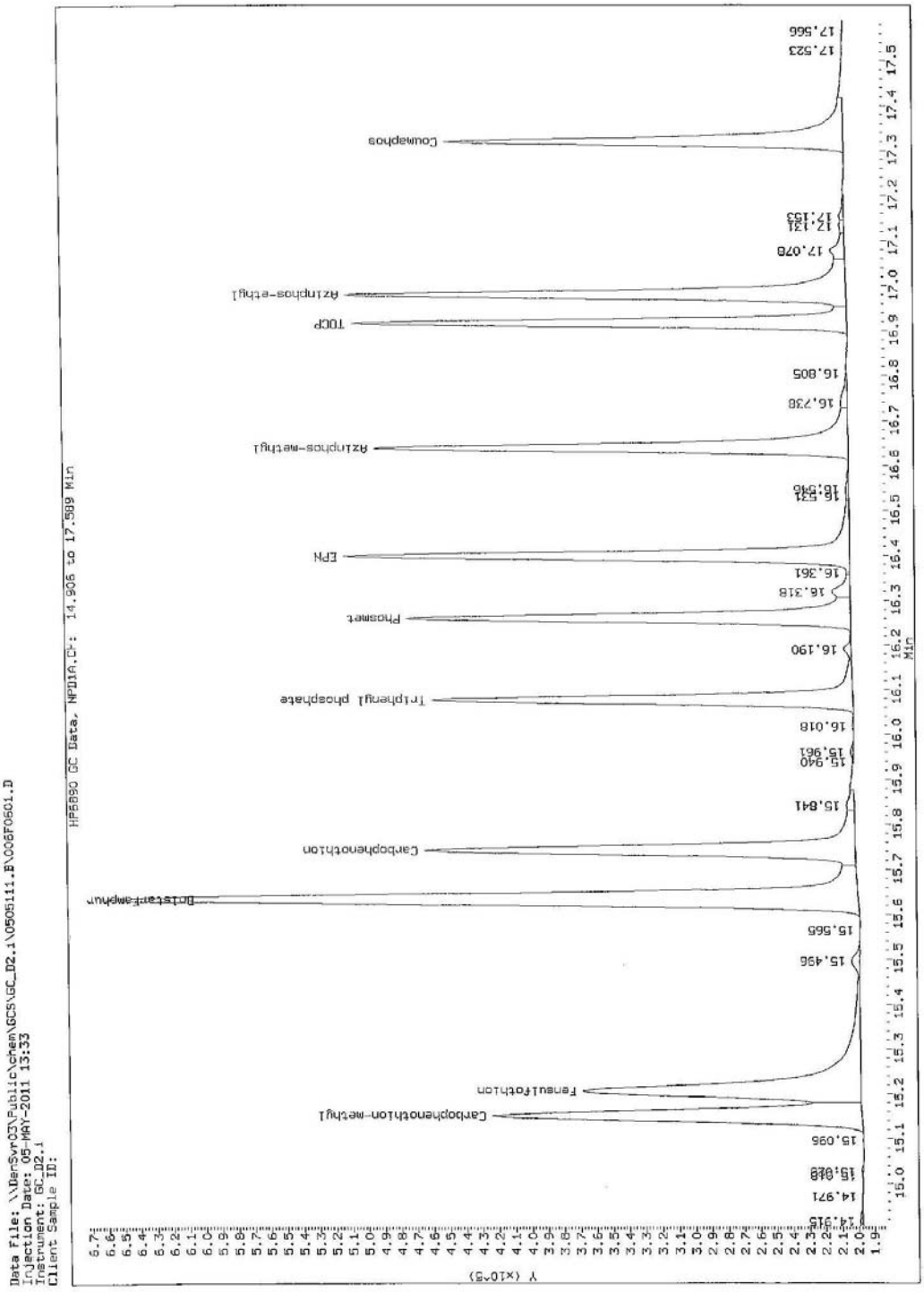
Attachment 2
Example Standard Chromatogram –Column: RTx-1MS
Expanded Views





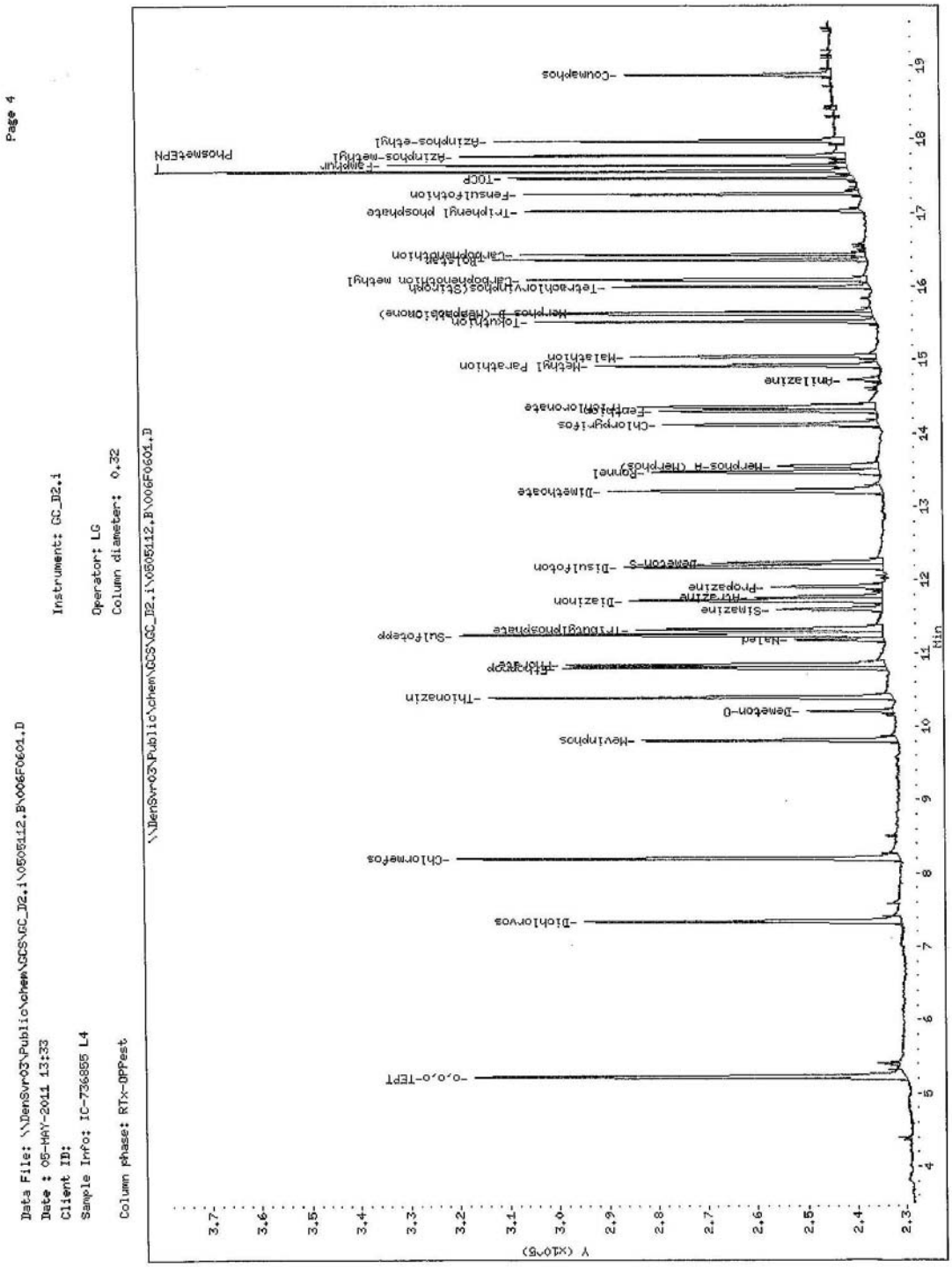
Note: Absolute retention times may vary due to actual column length and condition.

Attachment 2 (continued)
Example Standard Chromatogram –Column: RTx-1MS
Expanded Views



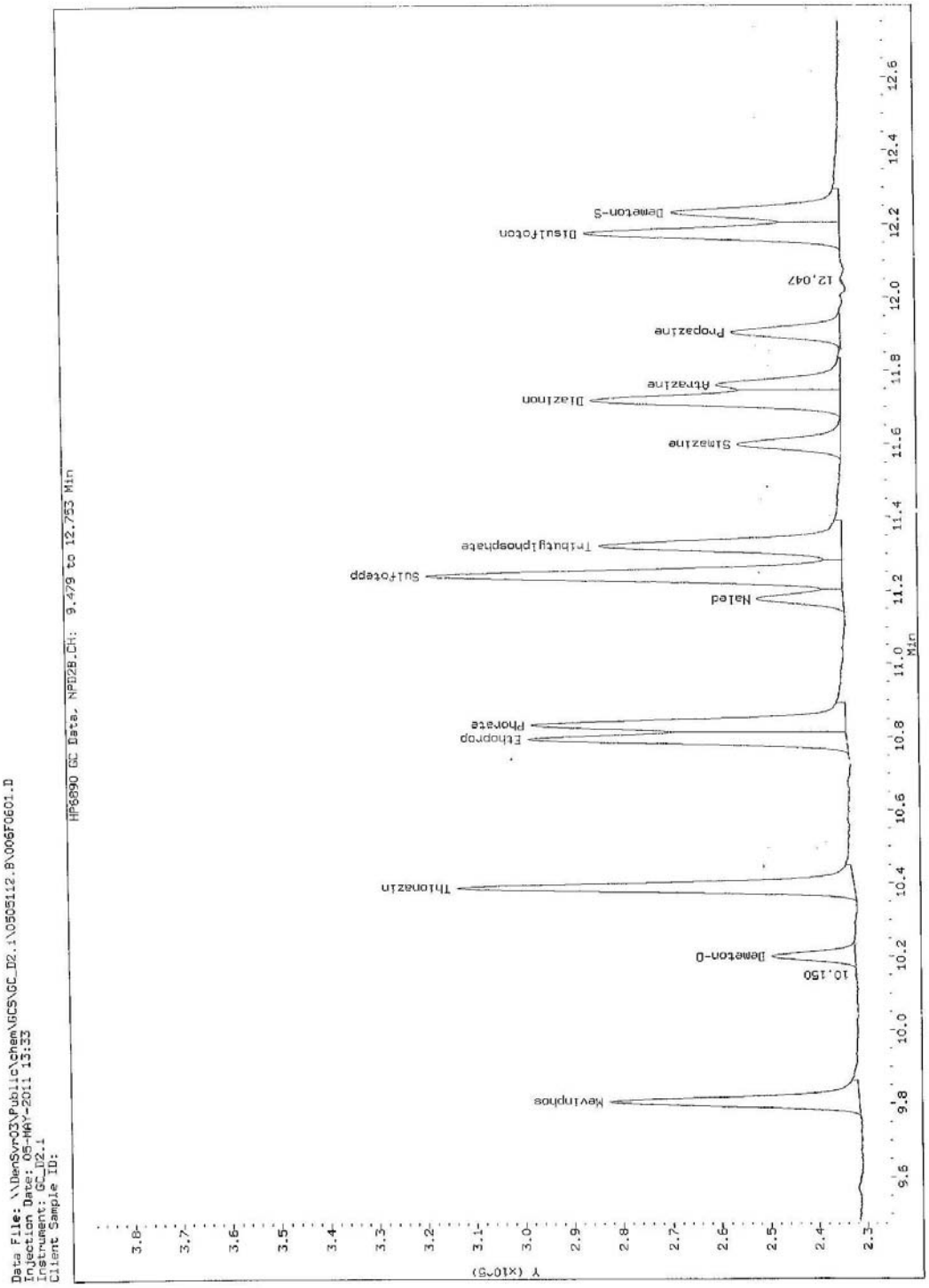
Note: Absolute retention times may vary due to actual column length and condition.

Attachment 3
Example Standard Chromatogram –Column: RTx-OPPest
Full Chromatogram



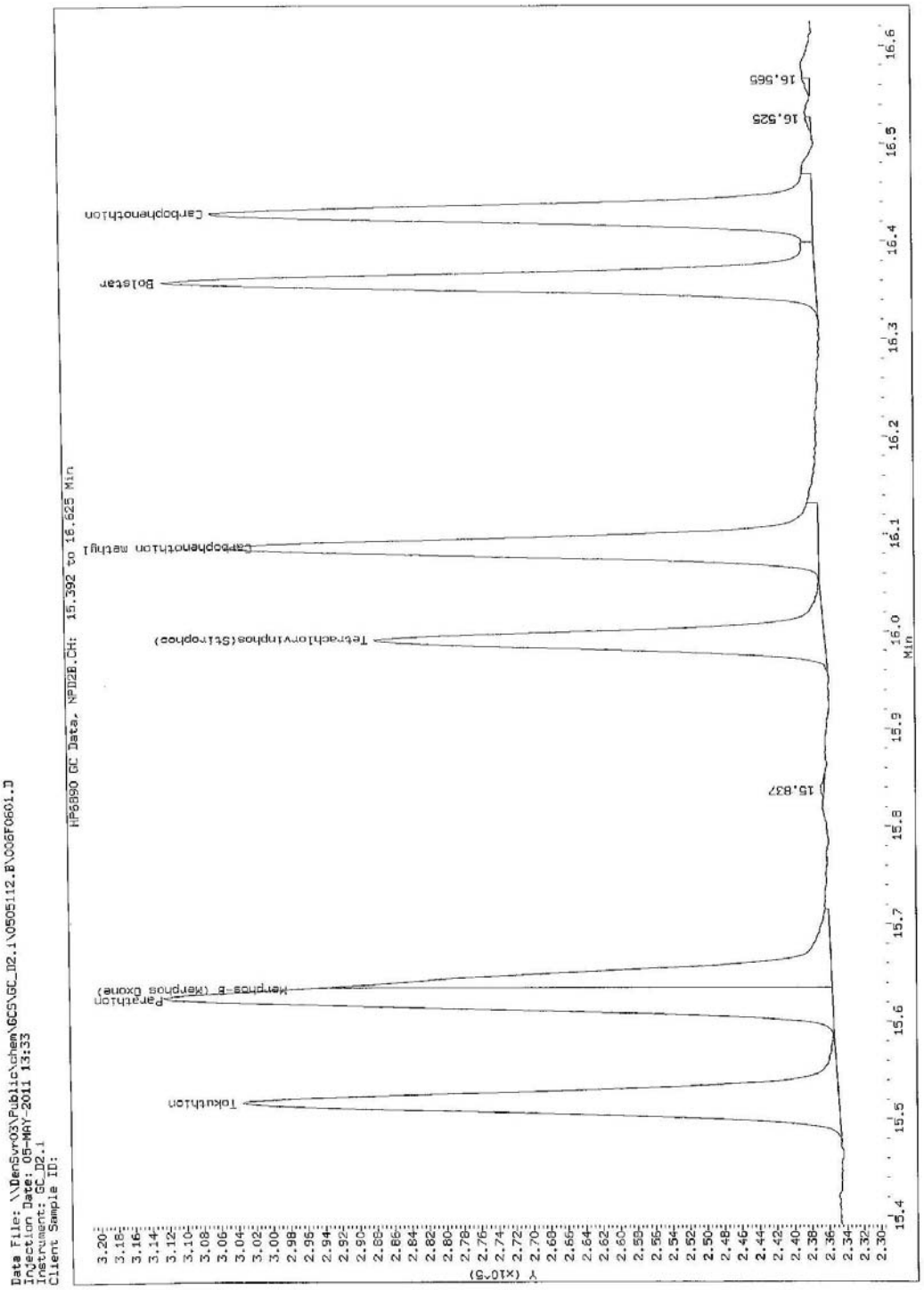
Note: Absolute retention times may vary due to actual column length and condition.

Attachment 4
Example Standard Chromatogram –Column: RTx-OPPest
Expanded Views



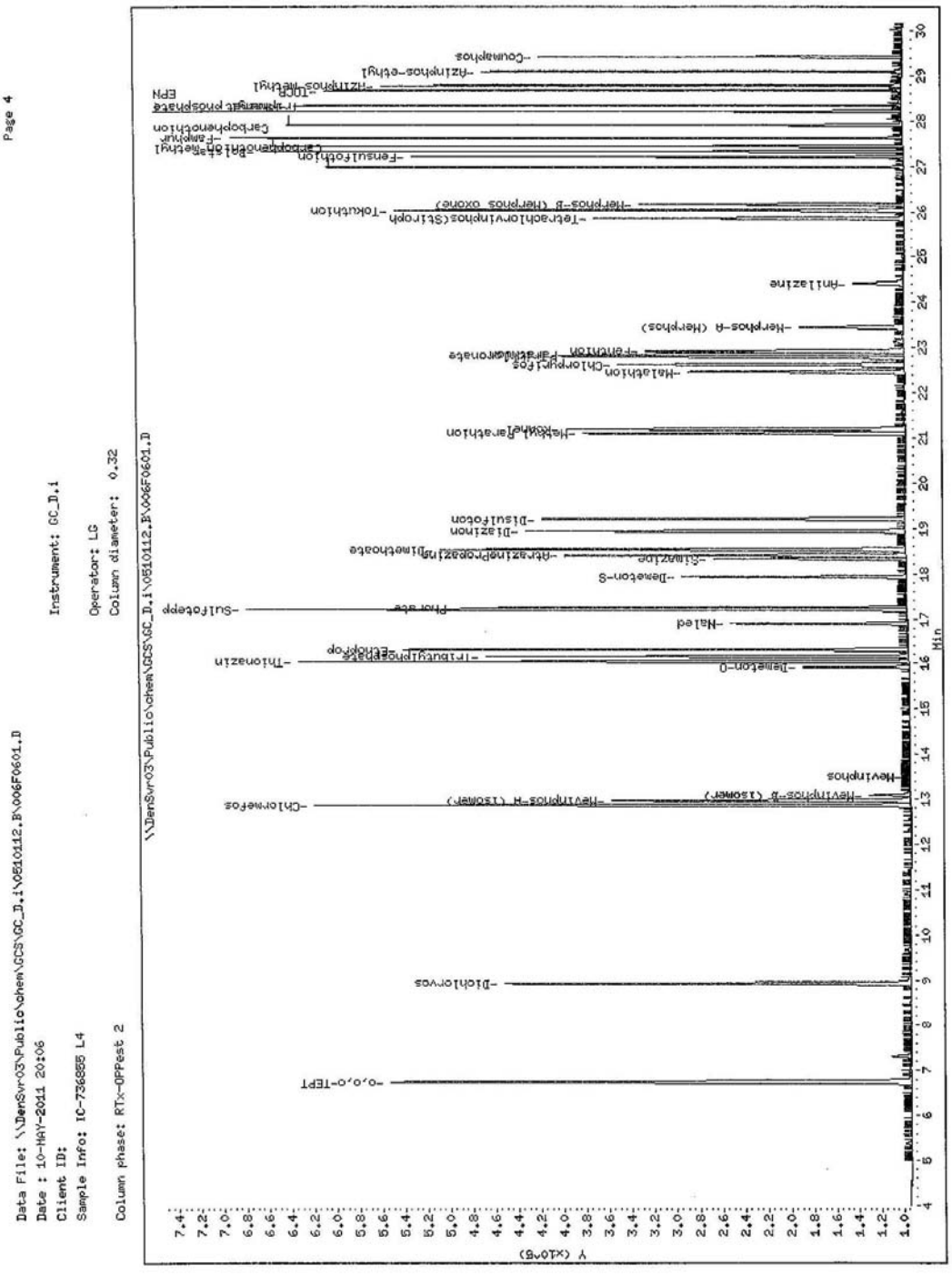
Note: Absolute retention times may vary due to actual column length and condition.

Attachment 4 (continued)
Example Standard Chromatogram –Column: RTx-OPPest
Expanded Views



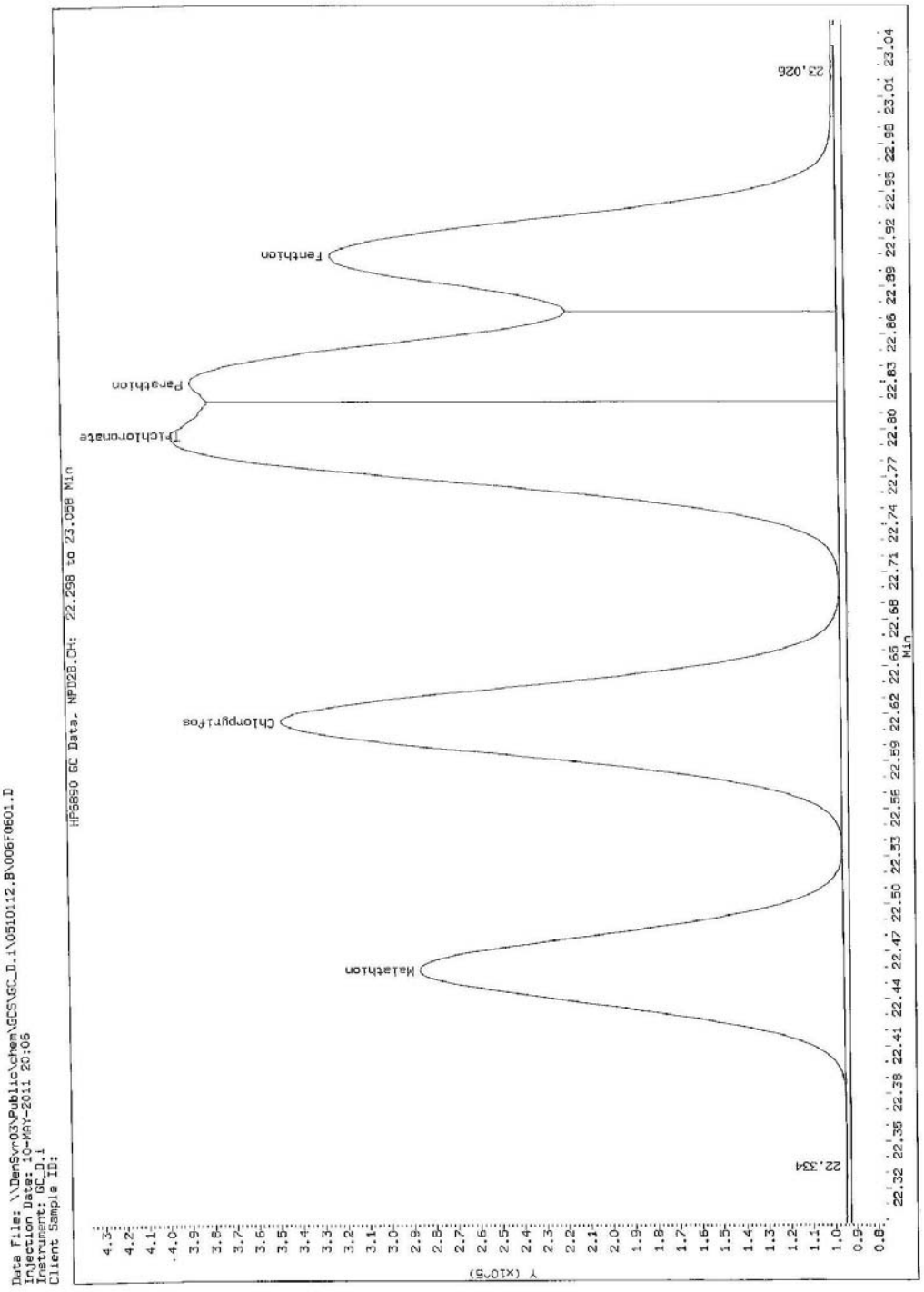
Note: Absolute retention times may vary due to actual column length and condition.

Attachment 5
Example Standard Chromatogram –Column: RTx-OPPest 2
Full Chromatogram



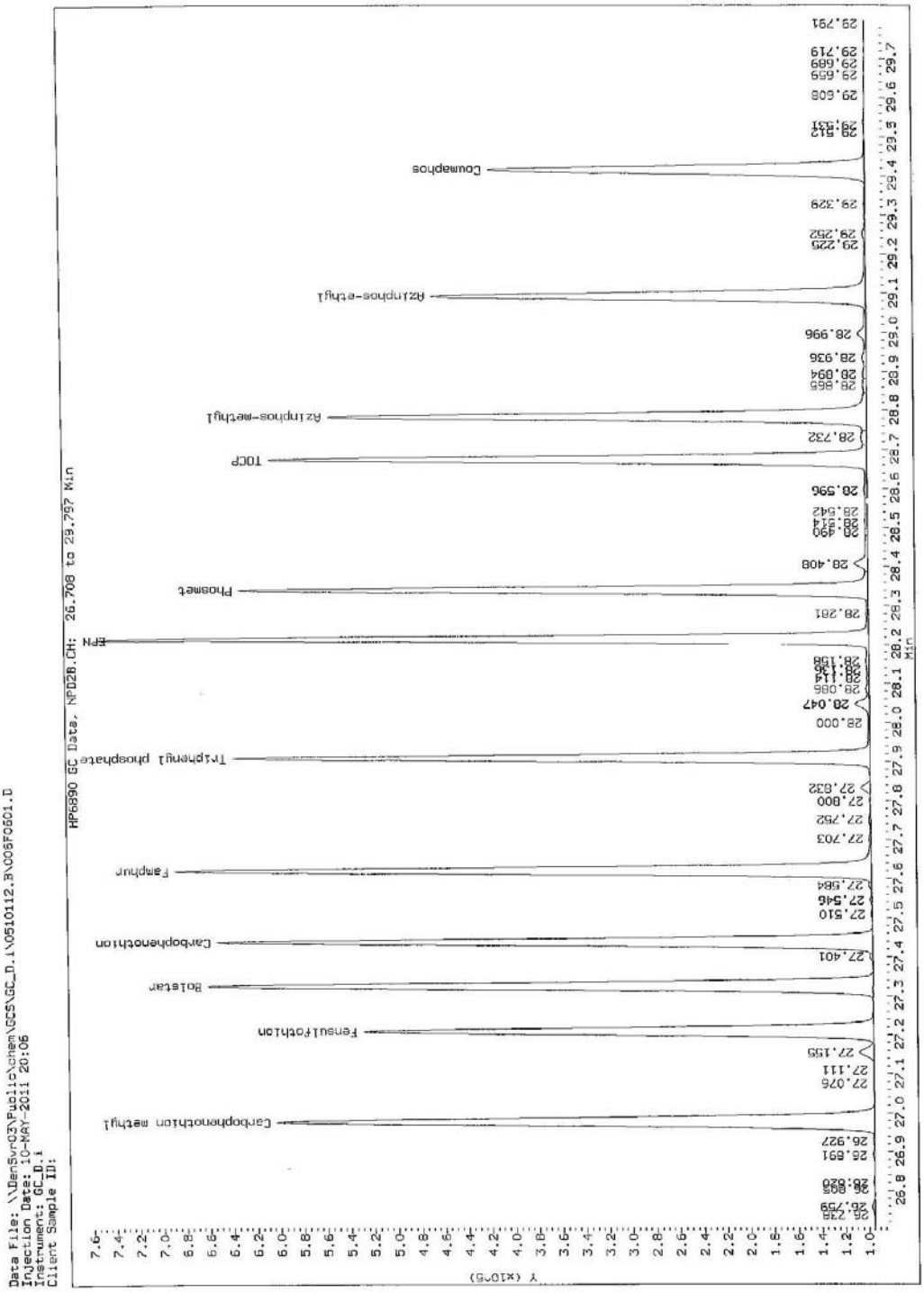
Note: Absolute retention times may vary due to actual column length and condition.

Attachment 6
Example Standard Chromatogram –Column: RTx-OPPest 2
Expanded Chromatograms



Note: Absolute retention times may vary due to actual column length and condition.

Attachment 6 (continued)
Example Standard Chromatogram –Column: RTx-OPPest 2
Expanded Chromatograms



Note: Absolute retention times may vary due to actual column length and condition.