METHOD 8000D

DETERMINATIVE CHROMATOGRAPHIC SEPARATIONS

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SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods in this manual, with the exception of required use for the analysis of method-defined parameters, are intended to be guidance documents. They contain general information on how to perform an analytical procedure or technique, which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. Performance data included in this method are for guidance purposes only and must not be used as absolute quality control (QC) acceptance criteria for the purpose of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

Please see Appendix A at the back of this document for a summary of revisions to Method 8000C (From Revision 3, March 2003).

1.1 Method 8000 is not a determinative method but instead provides guidance on analytical chromatography and describes calibration and QC requirements common to all SW-846 chromatographic methods. However, more specific QC requirements provided in the applicable determinative method will supersede those noted in Method 8000. Method 8000 should be applied in conjunction with all SW-846 determinative chromatographic methods. The methods include, but are not limited to, the following:

Method Number	Analytes	Chromatographic Technique (Sec. 1.5)	Detector
6850	Perchlorate	HPLC	MS, MS/MS
6860	Perchlorate	IC	MS, MS/MS
7580	White phosphorus (P_4)	GC	NICI/MS
8011	EDB, DBCP	GC, capillary column	ECD
8015	Non-halogenated volatiles	GC, packed & capillary column	FID
8021	Volatiles	GC, capillary column	PID, ELCD
8031	Acrylonitrile	GC, packed column	NPD
8032	Acrylamide	GC, packed column	ECD
8033	Acetonitrile	GC, capillary column	NPD
8041	Phenols	Underivatized or derivatized; GC, capillary column	FID, ECD
8061	Phthalates	GC, capillary column	ECD
8070	Nitrosamines	GC, packed column	NPD, ELCD, TED
8081	Organochlorine pesticides	GC, capillary column	ECD, ELCD
8082	Polychlorinated biphenyls	GC, capillary column	ECD, ELCD
8091	Nitroaromatics and cyclic	GC, capillary column	ECD
8100	Polynuclear aromatic hydrocarbons	GC, packed & capillary column	FID
8111	Haloethers	GC, capillary column	ECD
8121	Chlorinated hydrocarbons	GC, capillary column	ECD
8131	Aniline and selected	GC, capillary column	NPD
8141	Organophosphorus pesticides	GC, capillary column	FPD, NPD, ELCD
8151	Acid herbicides	Derivatized; GC, capillary column	ECD
8260	Volatiles	GC, capillary column	MS
8261	Volatiles	GC, capillary column	MS
8265	Volatiles	NA	DS/ITMS
8270	Semivolatiles	GC, capillary column	MS
8275	Semivolatiles	Thermal extraction/GC	MS
8276	Toxaphene and Congeners	GC, capillary column	NICI/MS
8280	Dioxins and Dibenzofurans	GC, capillary column	Low resolution MS
8290	Dioxins and Dibenzofurans	GC, capillary column	High resolution MS
8310	Polynuclear aromatic hydrocarbons	HPLC, reverse phase	UV, Fluorescence

Method Number	Analytes	Chromatographic Technique (Sec. 1.5)	Detector
8315	Carbonyl compounds	Derivatize; HPLC	UV
8316	Acrylamide, acrylonitrile, acrolein	HPLC, reverse phase	UV
8318	N-Methyl carbamates	Derivatize; HPLC	Fluorescence
8321	Extractable non-volatiles	PLC, reverse phase	TS/MS, UV
8323	Organotin compounds	HPLC, reverse phase	ES/ITMS
8325	Extractable non-volatiles	HPLC, reverse phase	PB/MS, UV
8330	Nitroaromatics and nitramines	HPLC, reverse phase	UV
8331	Tetrazene	HPLC, ion pair, reverse	UV
8332	Nitroglycerine	HPLC, reverse phase	UV
8410	Semivolatiles	GC, capillary column	FT-IR
8430	Bis(2-chloroethyl) ether hydrolysis products	GC, capillary column	FT-IR

DBCP = Dibromochloropropane
DS/ITMS = Direct sampling/ion trap mass spectrometry
ECD = Electron capture detector
EDB = Ethylene dibromide
ES/ITMS = Electrospray ionization/ion trap mass spectrometry
ELCD = Electrolytic conductivity detector
FID = Flame ionization detector
FPD = Flame photometric detector
FT-IR = Fourier transform-infrared
GC = Gas chromatography
HPLC = High performance liquid chromatography
MS = Mass spectrometry
MS/MS = Mass spectrometry/Mass spectrometry
NICI/MS = Negative Ion Chemical Ionization/Mass spectrometry
NPD = Nitrogen/phosphorous detector
NA = Not applicable
PAHs = Polynuclear aromatic hydrocarbons
PB/MS = Particle beam mass spectrometry
PID = Photoionization detector
TED = Thermionic emission detector
TS/MS = Thermospray mass spectrometry
UV = Ultraviolet

1.2 Analytical chromatography is used to separate target analytes from co-extracted interferences in samples. Chromatographic methods can be divided into two major categories: GC and HPLC.

1.2.1 GC is the separation technique of choice for organic compounds which can be volatilized without being decomposed or chemically rearranged.

1.2.2 HPLC is a separation technique useful for semivolatile and non-volatile chemicals or for analytes that decompose upon heating. Successful liquid chromatographic separation requires that the analyte(s) of interest be soluble in the solvent(s) selected for use as the mobile phase.

1.3 All chromatographic processes achieve separation by passing a mobile phase over a stationary phase. Constituents in a mixture are separated because they partition differently between the mobile and stationary phases and thus have different retention times. Compounds that interact strongly with the stationary phase elute slowly (i.e., longer retention times), while compounds that remain in the mobile phase elute quickly (i.e., shorter retention times).

1.3.1 The mobile phase for GC is an inert gas, usually hydrogen or helium, and the stationary phases are generally polymer bases.

1.3.2 In "normal phase" HPLC, the mobile phase is less polar than the stationary phase. In "reverse phase" HPLC, the converse is true. Reverse phase HPLC is the technique of choice for environmental and waste analyses of non-volatile organic target analytes.

1.3.3 Ion exchange chromatography is used to separate ionic species through competition with ions in the mobile phase for oppositely charged exchange sites on a stationary phase. Differential selectivities of the ionic species and the mobile phase ions for exchange sites are responsible for the chromatographic separation of the ions.

1.4 A number of specific GC and liquid chromatography (LC) techniques are used for environmental and waste analyses. Specific techniques are distinguished by the chromatographic hardware and chemical mechanisms used to achieve separations.

1.4.1 GC methods, including those in SW-846, can be categorized on the basis of the chromatographic columns employed.

1.4.2 HPLC methods in SW-846 are categorized on the basis of the mechanism of separation.

1.5 SW-846 methods describe columns and conditions that have been demonstrated to provide optimum separation of all or most target analytes listed in that specific procedure. Most often, those columns were the ones used by EPA during method development and testing. Analysts may change those columns and conditions, provided that they demonstrate performance for the analytes of interest that is appropriate for the intended application. This is especially true when limited groups of analytes are to be monitored (i.e., if only a subset of the list of target analytes in a method are needed, the chromatographic conditions and columns may be optimized for those analytes).

1.5.1 Chromatographic performance is demonstrated by the resolution of standards and the ability to model the response of the detector during calibration, and by sensitivity, precision, bias, frequency of false positives, and frequency of false negatives during analysis. The laboratory must demonstrate that any chromatographic procedure it uses provides performance satisfying the analytical requirements of the specific application for which it is being used. Such demonstrations should be performed using the procedures outlined in Secs. 9.2 to 9.8 of this method and appropriate sections in Chapter One.

1.5.2 Laboratories must also be cautious whenever the use of two dissimilar columns is included in a method for confirmation of identification and quantitation. For instance, a DB-5 column generally cannot be used for confirmation of results obtained using an SPB-5 column because the stationary phases are not sufficiently dissimilar and the changes in elution order (if any) will not provide adequate confirmation.

1.6 When GC conditions are changed, retention times and analytical separations are often affected. For example, increasing the oven temperature changes the rate of partitioning between the mobile and stationary phases, leading to shorter retention times. GC retention times can also be changed by selecting a column with a different length, stationary-phase loading (i.e., capillary column film thickness or percent loading for packed columns), or alternative liquid phase. As a result, two critical aspects of any SW-846 chromatographic method are the determination and/or verification of retention times and analyte separation.

1.7 HPLC retention times and analytical separations are also affected by changes in the mobile and stationary phases. The HPLC mobile phase is easily altered by adjusting the composition of the solvent mixture being pumped through the column. In reverse phase HPLC, increasing the ratio of water-miscible organic solvent to water generally shortens retention times. HPLC retention times can also be changed by selecting a column with a different length, alternative bonded phase, or dissimilar particle size (e.g., smaller particles and/or a longer column generally increase column resolution, while different bonded phases may resolve specific components differently). HPLC methods are also particularly sensitive to small changes in chromatographic conditions, including temperature. HPLC column temperature control ovens should be used to maintain constant retention times because ambient laboratory temperatures may fluctuate throughout the day. SW-846 methods provide conditions that have been demonstrated to provide good HPLC separations using specific instruments to analyze a limited number of samples. Analysts (particularly those using HPLC/MS) may need to tailor the chromatographic conditions listed in the method for their specific application and/or instrument.

1.8 Chromatographic methods can be used to produce data of appropriate quality for the analysis of environmental and waste samples. However, data quality can be greatly enhanced when the analyst understands both the intended use of the results and the limitations of the specific analytical procedures employed. Therefore, these methods are recommended for use only by, or under the close supervision of, experienced analysts. Many difficulties observed in the performance of SW-846 methods for the analysis of RCRA wastes can be attributed to the lack of skill and training of the analyst.

1.8.1 Methods using selective (e.g., PID, NPD, ELCD) or non-selective (e.g., FID) detectors may present serious difficulties when used for site investigations, including coelution of target analytes, false negatives due to retention time shifts, and false positives and quantitation errors due to coeluting non-target sample components.

1.8.2 In contrast, GC methods employing selective or non-selective detectors may be appropriate for remediation activities where the analytes of concern are known, of limited number, and of significantly greater concentration than potentially interfering materials.

1.8.3 If the site is not well characterized, and especially if large numbers of target analytes are of concern, analysis by GC/MS or HPLC/MS may be more appropriate.

1.9 Each chromatographic method includes a list of the compounds recommended for analysis given the procedures described therein. Lists in some methods are lengthy; it may not be practical or appropriate to determine all the analytes simultaneously. Such analyte lists do not imply a regulatory requirement for the analysis of any or all of the compounds, but rather indicate the method(s) applicable to those compounds.

1.10 Analysts should consult the disclaimer statement at the front of the manual and the information in Chapter Two for 1) guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and 2) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrices of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly required in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance for the analyst and regulated community in making judgments necessary to generate results meeting the data quality requirements for the intended application.

1.11 All of the SW-846 determinative chromatographic methods that reference this method are restricted to use by, or under the supervision of, analysts experienced in the use of gas or high-performance liquid chromatographs and skilled in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate an acceptable initial demonstration of proficiency (IDP) along with acceptable results according to method recommendations and stated project data quality objectives (DQOs). Method 8000 is intended to be a supplement to, but is not intended to be a substitute for, formal training in the basic principles of GC, GC/MS, LC, LC/MS or HPLC.

2.0 SUMMARY OF METHOD

This method describes general considerations in achieving chromatographic separations and performing calibrations. It is to be used in conjunction with all SW-846 determinative chromatographic methods, including, but not limited to, each method listed in Sec. 1.1. Each of these chromatographic methods recommends appropriate procedures for sample preparation, extraction, cleanup, and/or derivatization. Consult the specific procedures for additional information on these crucial steps in the analytical process.

2.1 Sec. 4.2 of this method provides general guidance on minimizing contamination, including cross-contamination between samples. Sample screening procedures are strongly recommended and discussed in Sec. 4.3.

2.2 Before any sample or blank is introduced into a chromatographic system, the appropriate resolution criteria and calibration procedure(s) described in Method 8000 must be satisfied.

2.3 Secs. 4.4 and 4.5 provide information on the effects of chromatographic interferences.

2.4 Sec 6.0 of this method contains generalized specifications for the components of both GC and HPLC systems used in SW-846 analyses.

2.5 Calibration of the analytical system is another critical step in the generation of quality data. Sec. 11.5 discusses specific procedures and calculations for both linear and non-linear calibration models. Continued use of any chromatographic procedure necessitates a verification of the calibration model, and procedures for such verifications are described in this method as well (Sec. 11.7).

2.6 Identification of target compounds by any chromatographic procedure is based, at least in part, on retention times. Sec. 11.6 provides procedures for the determination of retention times and retention time windows to be used with the specific methods listed in Sec. 1.1.

2.7 Calculations necessary to derive sample-specific concentrations from the instrument responses are common to most of the analytical methods listed in Sec. 1.1. Commonly used calculations are summarized in Sec. 11.10.

2.8 Preventive maintenance and corrective actions are essential to the generation of quality data in a routine laboratory setting. Suggestions for such procedures are found in Sec. 11.11.

2.9 Most of the methods listed in Sec. 1.1 employ a common approach to QC. While some of the overall procedures are described in Chapter One, Sec. 9.0 describes routinely used procedures for calibration verification, instrument performance checks, demonstrating acceptable performance, etc.

2.10 Before performing analyses of specific samples, analysts should work with data users to determine acceptable recovery ranges for all target analytes of interest in the type of matrices to be tested. Analysts must also be able to demonstrate that the sensitivity of the procedure employed is appropriate for the intended application. One approach to such a demonstration is to estimate the method sensitivity for the analytes of interest using the procedures in Chapter One or other appropriate procedures.

3.0 DEFINITIONS

Refer to Chapter One, the individual determinative methods, and the manufacturer's instructions for definitions that may be relevant.

4.0 INTERFERENCES/CHROMATOGRAPHIC PERFORMANCE

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences during sample analysis. All of these materials must be demonstrated to be free from interferences under conditions of analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on QC procedures and to Chapter Four for general guidance on the cleaning of glassware.

4.2 Contamination by carryover can occur whenever high- and low-concentration samples are analyzed in sequence. To reduce potential for carryover, the sample syringe or purging device must be thoroughly rinsed between samples with an appropriate solvent (including water). Purge-and-trap or headspace devices should be thoroughly baked out between samples.

Where practical, samples with unusually high concentrations of analytes should be followed by method blanks, instrument blanks, or by analysis of organic-free reagent water to check for carryover contamination. If target compounds present in an unusually highly concentrated sample are also found to be present in subsequent samples, the analyst must demonstrate that the compounds are not affected by carryover contamination. Conversely, if those target compounds are <u>not</u> present in the subsequent sample(s), then they do not need to be reanalyzed.

Purging vessels may be cleaned by rinsing with methanol, followed by a distilled water rinse and drying in a 105 °C oven between analyses. Detergent solutions may also be used, but care must be taken to remove the detergent residue from the purging vessel. Other approaches to cleaning purging vessels, such as some modern autosamplers which rinse the vessel(s) between runs, may also be employed, provided that the laboratory can demonstrate that they are effective in removing contaminants.

4.3 In addition to carryover of compounds from one sample to the next, the analysis of high-concentration samples can lead to contamination of the analytical instrument itself. Eliminating this contamination can cost significant time and effort that cannot be spent analyzing samples. The most reliable procedure for ensuring minimum down time is to screen samples

by a higher level technique. Samples to be analyzed for volatiles can be screened using an automated headspace sampler (Method 5021) connected to a GC/PID/ELCD detector (Method 8021) or by analyzing large (e.g., 100-fold) dilutions of the samples on the GC/MS. Samples to be analyzed for semivolatiles can be screened using GC/FID. Other screening methods are also acceptable. The analyst should use screening results to choose an appropriate dilution factor for the GC/MS analysis that will prevent system contamination yet still provide adequate sensitivity for the major constituents of the sample.

4.4 Elevated chromatographic baselines (e.g., baseline humps) should be minimized or eliminated during these analyses by application of appropriate sample clean-up (Method 3600), extract dilution, use of pre-columns and/or inserts, or employing a selective detector. Integration of “hump-o-grams” can result in significant quantitative errors. When elevated baselines are observed during analysis of blanks and standards, the chromatographic system should be considered contaminated. This contamination can result from impure carrier gas, inadequate gas conditioning, septum bleed, column oxidation, incomplete elution of non-target interferences, and/or pyrolysis products in the injector or column. Such contamination is unacceptable and must be addressed through a program of preventive maintenance and corrective action.

4.5 See Sec. 11.11 for suggested preventative maintenance activities that may prevent or ameliorate deterioration of chromatographic performance.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals and instrumentation included in this method. A reference file of safety data sheets (SDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

6.1 Mention of trade names or commercial products in this manual is for illustrative purposes only and does not constitute an EPA endorsement or exclusive recommendation for use. Products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list all common laboratory glassware (e.g., beakers and flasks) that might be used.

6.2 GC inlet systems

6.2.1 Volatile organics - Volatile organic analytes are introduced into a GC through a purge-and-trap system, by direct injection, or other technologies. The purge-and-trap apparatus is described in Method 5030 for water samples and in Method 5035 for soil and other solid samples. See Method 5000 for guidance on all forms of sample introduction of volatiles into the GC and GC/MS system.

6.2.2 Semivolatile organics - Sample extracts containing semivolatile organic compounds are introduced into a GC with a syringe that passes through a septum into an injection port. The injection port allows the sample extract to be vaporized prior to being flushed onto the GC column, hence the term “gas” chromatography. Correct setup and maintenance of the injector port is necessary to achieve acceptable performance with GC methods.

6.2.2.1 An injection port septum and liner should be installed in the GC inlet as appropriate for the system.

6.2.2.2 Packed columns and wide-bore capillary columns (> 0.32-mm ID) should be mounted in ¼-inch injectors.

6.2.2.3 Narrow-bore capillary columns (≤ 0.32 mm ID) should be mounted in split/splitless (Grob-type) injectors. Split/splitless injectors should have automated valve closures that direct most of the flow (and sample) onto the head of the analytical column. After a predetermined splitless introduction time, the split valve is opened so that most of the flow is vented during analysis, thus eliminating the solvent tail while maintaining proper flow through the column. The initial oven temperature should be below the boiling point of the injection solvent if the solvent front interferes with early-eluting analytes or if the solvent effect is needed to resolve difficult to separate analytes.

6.2.2.4 Cool on-column injection and programmable temperature vaporizer inlets allow the analysis of labile compounds that degrade on packed columns and in split/splitless injectors.

6.3 GC flow control

Precise control of the gas mobile phase is necessary to achieve reproducible GC retention times. Flow controllers within any GC used for analyses described in SW-846 methods should deliver a precisely metered gas flow at a rate appropriate for the GC column mounted in the instrument.

6.3.1 Most GCs have restrictors built into electronic pressure controllers (EPCs) monitored using a digital readout. These restrictors are used to provide precise flow at the carrier gas flow rate listed in the method (e.g., use <20 mL/min restrictors for wide-bore capillary methods).

6.3.2 Analysts should ensure that cylinder pressures are regulated properly and manifold pressures are sufficiently large such that a change in the head pressure of an individual instrument does not affect the flow through all instruments. Toggle valves that allow instruments to be isolated are recommended for all multi-instrument gas delivery systems.

6.3.3 Carrier gas should be of high purity and conditioned between the cylinder and the GC with a scrubber to remove any residual water, oxygen and hydrocarbons as necessary. Gas regulators should contain stainless steel diaphragms. (Neoprene diaphragms are a potential source of gas contamination and should not be used.)

6.4 GC columns - Each determinative method in SW-846 provides a description of a chromatographic column or columns with associated column specifications. Other GC columns may be substituted in SW-846 methods to improve performance if (1) the criteria described in

Sec. 9.3 are satisfied, and (2) target analytes are sufficiently resolved from one another and from co-extracted interferences to provide data of appropriate quality for the intended application.

Use of capillary columns has become standard practice in environmental and waste analysis. Capillary columns have an inherently greater ability to separate analytes than packed columns. However, packed columns can provide adequate resolution of some analytes and are most appropriately employed when the list of analytes to be determined is relatively short.

6.4.1 Narrower columns are more efficient (i.e., can resolve more analytes) but have a lower capacity (i.e., can accept less sample without peak distortion).

6.4.2 Longer columns can resolve more analytes; resolution increases as a function of the square root of column length. Run-time is also increased.

6.4.3 Columns with greater film thickness (i.e., loading) increases column capacity and retention times.

6.5 GC detectors

Detectors are the transducers that respond to components eluting from a GC column and produce the electrical signal used for quantitative determinations. SW-846 analyses in this manual are conducted using the detectors listed in Sec. 1.1. Except where otherwise recommended by the instrument manufacturer, selective non-MS detectors should be maintained at least 20 °C above the highest oven temperature employed to prevent condensation and detector contamination. To prevent condensation between the GC and an MS detector, transfer lines should be maintained at a temperature above the highest temperature of the oven program, or as specified by the instrument manufacturer.

6.6 HPLC injectors

Liquids are essentially uncompressible, so a mechanical device is necessary that allows introduction of the sample into a high-pressure flow without significant disruption in the flow rate and hydraulic pressure. Normally, a 6-port valve is used for this purpose. A sample loop is isolated from the flow of the mobile phase and filled with a sample extract. (Larger sample loops may be used to increase sensitivity; however, they may degrade chromatographic performance). The extract is then injected by the valve being turned so that the mobile phase flows through the loop. This procedure virtually eliminates dead volume in the injector and is fully compatible with automated operation.

6.7 HPLC pumps

The mobile phase used for HPLC should be accurately pressurized before it enters the injector. HPLC pumps are generally capable of delivering solvent at 5000 psi or above with excellent precision. Rate of delivery depends on the column used for the separation. Flow rates should be checked by collecting column effluent in a graduated cylinder for a designated time period.

Most pumping systems are capable of changing solvent concentration during an analysis (i.e., gradient elution). Gradients are generated by either high pressure mixing of two streams between the pump and the injector or by proportional mixing of the solvents before they are pumped. In either case, solvent mixing can cause changes in the solubility of dissolved gases, formation of bubbles in the mobile phase, or non-reproducible gradients.

6.8 HPLC Columns

HPLC columns are generally constructed of stainless steel tubing and are sealed with compression fittings. These columns should be constructed with minimum dead volume and a narrow particle size distribution. Manufacturers provide columns bonded with dissimilar functional groups (e.g., C_{18}, cyano, TMS) and have different percent carbon loading.

6.8.1 Use of high quality columns that are uniformly packed with the appropriate particle size and bonded phase will result in optimal chromatographic performance. For example, columns with silica-based particles with free silol groups show less tailing of polar materials (e.g., amines).

6.8.2 A smaller particle (and pore) size generally gives better resolution, higher back pressure, and smaller sample capacity.

6.8.3 Lifetime and performance of HPLC columns can be improved through proper maintenance. Analysts should filter sample extracts and use compatible guard columns.

6.9 HPLC column temperature control ovens

HPLC retention times are more reproducible if the column is held at a constant temperature. Temperature control ovens capable of maintaining the HPLC column at ± 0.1 °C should be utilized to provide consistent retention times throughout the course of an HPLC analysis. Normal oven operating temperature should be 3 – 5 °C above ambient laboratory temperature.

6.10 HPLC detectors

Detectors are the transducers that respond to components eluting from a HPLC column and produce the electrical signal used for qualitative and quantitative determinations. SW-846 analyses are conducted using selective detectors or mass spectrometers listed in Sec. 1.1. HPLC/MS involves the use of a sophisticated interface that separates target analytes from the aqueous mobile phase. Examples include the thermospray (TSP), electrospray (ESP), and the atmospheric pressure chemical ionization (APCI) interfaces.

6.11 Data systems

Raw chromatographic data have to be reduced in order to provide the quantitative information needed by analysts. Sophisticated data systems are strongly recommended for SW-846 chromatographic methods because the ability to store and re-plot chromatographic data is invaluable during data reduction and review. Organizations should select the system most suitable for their applications.

6.12 Supplies

Chromatographers use a variety of supplies. Specific items that should be stocked depend on laboratory instrumentation and the analyses performed. At a minimum, laboratories need PTFE tape, stainless steel regulators, acid-washed copper tubing, syringes, and replacement parts for instruments.

6.12.1 Laboratories performing GC analyses also need supplies such as high purity gases, scrubbers for gas conditioning, gas-tight fittings, capillary cutters, magnifying glasses, septa with proper temperature limits, appropriate ferrules, dichlorodimethylsilane (for deactivating surfaces), glass wool, spare columns and injection port liners.

6.12.2 Laboratories performing HPLC analyses need supplies such as high purity solvents, column packing material, frits, narrow inner diameter tubing, appropriate ferrules, solvent filtration apparatus, and solvent degassing equipment.

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent leaching of contaminants from plastic containers.

7.2 See specific extraction and determinative methods for the reagents and standards needed.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, they must be followed. In the absence of specific regulatory requirements, use the information in Chapter Four as guidance in determining sample collection, preservation, and storage requirements. Additional information may be found in some of the individual sample extraction, preparation, and determinative methods.

9.0 QUALITY CONTROL

9.1 General Guidance

Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific and Chapter One criteria; technique-specific QC criteria take precedence over Chapter One criteria. Any effort involving collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged, as described in Sec. 9.6. Use of instrument–specific QC limits is encouraged, provided such limits will generate data appropriate for use in the intended application. All data sheets and QC data should be maintained for reference or inspection.

9.2 Evaluating chromatographic performance

The analyst's expertise in performing chromatography is a critical element in the successful performance of chromatographic methods. Successful generation of data demands selection of suitable preparative and determinative methods and an experienced staff to use these methods.

9.2.1 For each 12-hour period during which analysis is performed, the performance of the instrument system should be checked. These checks should be part of a formal QC program that includes analysis of instrument blanks, calibration standards, and other QC as appropriate for that method. In addition to these instrument QC checks, performance of the entire analytical process (i.e., preparation, cleanup and analysis) should be monitored. These additional checks should include method blanks, matrix spikes/matrix spike duplicates (MS/MSD), laboratory control samples (LCS), replicate samples and other QC as appropriate for that method or project. It is generally advisable, although not required, that all method QC samples be run at the same time as the samples on the same instrument.

9.2.2 Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method and/or the measurement quality objectives (MQOs) established for intended application.

9.2.3 In addition to the quantitative measures of comparison described below and in the individual methods, analysts should evaluate chromatograms and instrument operation. Questions that should be asked include the following:

Do the peaks look normal (Gaussian)?
Is the response obtained comparable to the response from previous calibrations?
Are non-target peaks present in calibration analyses?
Are contaminants present in the blanks?
Is the injector leaking (e.g., does the GC injector septum need replacing)?
Do the column fittings need tightening?
Does the HPLC guard column need replacement?

9.2.4 Significant peak tailing, leaks, changes in detector response and laboratory contamination should be corrected. Tailing problems are generally traceable to active sites on the column, cold spots in a GC, improper choice of HPLC mobile phase, the detector inlet, or leaks in the system.

9.2.5 Recalibration of the instrument must take place when performance changes to the point that the calibration verification acceptance criteria (Sec. 11.7) cannot be achieved. Recalibration of the instrument should be performed as required per determinative methods.

9.2.6 Before processing any samples, the analyst should demonstrate that all parts of the equipment contacting the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. Each time samples are extracted, cleaned up, and analyzed, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. Consult the appropriate 3500 or 5000 series method for specifics of the preparation of method blanks. The following general guidelines apply to the interpretation of method blank results.

9.2.6.1 Method blanks should be prepared at a frequency of at least 5%: one method blank for each group of up to 20 samples prepared at the same time, by the same procedures. For volatile samples analyzed by purge-and-trap, preparation is equivalent to the analysis. Therefore, one purge-and-trap method blank must be analyzed with each group of up to 20 samples analyzed on the same instrument during the same analytical shift.

9.2.6.2. When samples that are extracted together are analyzed on separate instruments or in separate analytical shifts, the method blank associated with those samples (e.g., extracted with the samples) must be analyzed on at least one of those instruments. A solvent blank should be analyzed on all other instruments on which the set of samples was analyzed to demonstrate the instrument is not contributing contaminants to the samples.

9.2.6.3 Unless otherwise described in a determinative method, the method blank may be analyzed immediately after the calibration verification standard to ensure that there is no carryover from the standard or at another point in the analytical shift.

9.2.6.4 When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures.

9.2.6.5 Results of the method blank should be less than the lower limit of quantitation (LLOQ) (Sec. 9.7) for the analyte or less than the level of acceptable blank contamination specified in the approved QAPP or other appropriate systematic planning document.

9.2.6.6 If the method blank results do not meet the acceptance criteria above, the laboratory should take corrective action to locate and reduce the source of the contamination and re-extract and reanalyze any samples associated with the contaminated method blank. If the method blank results still do not meet the acceptance criteria in 9.2.6.5 and re-analysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory. Qualification of the samples may be needed.

9.2.6.7 The laboratory should <u>not</u> subtract the results of the method blank from those of any associated samples. Such “blank subtraction” is inappropriate for the GC and HPLC methods addressed here and may lead to negative sample results.

9.2.6.8 Blanks – Before processing any samples, the analyst should demonstrate through the analysis of a method blank that equipment and reagents are free from contaminants and interferences. If a peak is found in the blank that would prevent the identification or bias the measurement of an analyte, the analyst should determine the source and eliminate it, if possible. As a continuing check, each time a batch of samples is extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. Method blanks, trip blanks, and other field blanks should be carried through all stages of sample preparation and analysis. At least one

method blank or instrument blank must be analyzed on every instrument after calibration standard(s) and prior to the analysis of any samples.

9.2.6.9 Blanks are generally considered to be acceptable if target analyte concentrations are less than one-half the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., targets are not present in samples or sample concentrations are ≥10X the blank). Other criteria may be used depending on the needs of the project.

9.2.6.10 If an analyte of interest is found in a sample in the batch near a concentration confirmed in the blank (refer to Sec. 9.5.2), the presence and/or concentration of that analyte should be considered suspect and may require qualification. Contaminants in the blank should meet most or all of the qualitative identifiers in Section 11.6 to be considered. Samples may require re-extraction and/or re-analysis if the blanks do not meet lab established or project specific criteria. Re-extraction and/or re-analysis is not necessary if the analyte concentration falls well below the action or regulatory limit or if the analyte is deemed not important for the project.

9.2.6.11 When new reagents or chemicals are received, the lab should monitor the blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.2.6.12 Method and/or solvent blanks may also be used to check for contamination by carryover from a high-concentration sample into subsequent samples (Sec. 4.2). When analysis of such blanks is not possible, such as when an unattended autosampler is employed, the analyst should carefully review the results for at least the next sample after the high-concentration sample. If analytes in the high-concentration sample are <u>not</u> present in the subsequent sample, then lack of carryover has been demonstrated. If there is evidence that carryover may have occurred, then the affected samples should be reanalyzed.

9.3 Initial demonstration of proficiency (IDP)

Prior to implementation of a method, each laboratory must perform an IDP consisting of at least four replicate reference samples spiked into a clean matrix taken through the entire sample preparation and analysis. Whenever a significant change to instrumentation or procedure occurs, the laboratory must demonstrate that acceptable precision and bias can still be obtained by the changed conditions (Sec. 9.3.1). Whenever new staff members are trained, an analyst IDP must be performed. (Sec. 9.3.2).

9.3.1 Demonstration of proficiency for instrument or method changes

If a major change to the sample preparation procedure is made (e.g., a change in solvent), the IDP must be repeated for that preparation procedure by a minimum of four spiked reference samples. Alterations in instrumental procedures only, such as changing GC temperature programs or HPLC mobile phases or the detector interface, require a new calibration but not a new IDP because the preparation procedure is unchanged. Each laboratory must have policy for performance and documentation of IDP.

9.3.2 Demonstration of proficiency for new analysts

Each laboratory should have a training program which documents that a new analyst is capable of performing the method, or portion of the method, for which the analyst is responsible. This demonstration should document that the new analyst is capable of successfully following the SOP established by the laboratory.

For example, when analysts are trained for a subset of analytes for an 8000 series method, the new sample preparation analyst should prepare reference samples for a representative set of analytes (e.g., the primary analyte mix for Method 8270, or a mix of Aroclor 1016 and 1260 for Method 8082) for each preparation method the analyst will be performing. The instrument analyst being trained will need to analyze prepared samples (such as semi-volatile extracts).

9.3.3 Preparation of reference samples

9.3.3.1 Reference samples are prepared from a spiking solution containing each analyte of interest. The reference sample concentrate (spiking solution) may be prepared from pure standard materials or purchased as certified solutions. This reference standard should be made from the same source as the calibration standards to eliminate any additional variability due to differences between sources.

9.3.3.2 Preparation of the reference sample concentrate is dependent upon the method being evaluated. Guidance for certain methods is listed in Methods 3500 and 5000. In other cases, the determinative methods contain guidance on preparing the reference sample concentrate and the reference sample. If no guidance is provided, prepare a reference sample concentrate in methanol (or any water-miscible solvent) at a concentration such that the spike will provide a concentration in the clean matrix near the middle of the calibration range for each analyte in that matrix.

9.3.3.3 Concentrations of target analytes in the reference sample may be adjusted to reflect more accurately the concentrations to be analyzed by the laboratory. If the concentration of an analyte is being evaluated relative to a regulatory limit or action level, see Sec. 9.4.1 for information on selecting an appropriate spiking level.

9.3.4 Evaluation

9.3.4.1 To evaluate the performance of the total analytical process, reference samples must be handled in exactly the same manner as actual samples. Additional LCS or MS/MSD samples can be omitted. Use a clean matrix for spiking purposes (one without any target or interference compounds) such as organic-free reagent water for aqueous matrices and organic-free sand or soil for solid matrices.

9.3.4.2 Prepare and analyze at least four replicate aliquots of the well-mixed reference samples by the same procedures used to analyze actual samples (procedure section for each of the methods). This will include a combination of the sample preparation method (usually a 3500 series method for extractable

organics or a 5000 series method for volatile organics) and the determinative method (an 8000 series method).

9.3.4.3 Calculate the mean recovery ($\bar{x}$) and the standard deviation of the recovery (*s*) for each analyte of interest using the four results.

9.3.4.4 Multiple-laboratory performance data are included in some determinative methods and may be used as guidance in evaluating performance in a single laboratory. However, comparison with single-laboratory performance data is much more indicative regarding expectations of how any individual laboratory will perform, than in comparison with multi-laboratory data. Compare *s* and $\bar{x}$ for each analyte with the corresponding performance data for precision and bias given in the performance table at the end of the determinative method. If *s* and ($\bar{x}$) for all analytes of interest meet the appropriate acceptance criteria, then the system performance is acceptable and analysis of actual samples can begin. If any individual s value exceeds the precision limit or any $\bar{x}$ value falls outside the range for bias, then the system performance may be unacceptable for that analyte. Once sufficient data points are available, each laboratory is strongly encouraged to develop in-house control limits.

NOTE: The large number of analytes in each of the methods presents a substantial probability that one or more analyte will fail at least one of the performance criteria when all analytes of a given method are determined.

9.3.4.5 Performance data in many of the methods are based on single-laboratory performance. As with multiple-laboratory data, the criteria in those methods may be used as guidance when evaluating laboratory performance. When comparing your laboratory data to performance data developed from single-laboratory data, certain analytes may be outside the limits; however, the majority should be within the acceptance limits.

9.3.4.6 When one or more of the analytes fail at least one of the performance criteria, the analyst should repeat the test only for those analytes that fail to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning at Sec. 9.3.4.

9.4 Matrix spike, laboratory control samples and method blanks

9.4.1 General Discussion

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision and bias). At a minimum, this check should include the analysis of at least one matrix spike and one duplicate unspiked sample or preferably, one MS/MSD pair with each preparation batch of up to 20 samples of the same matrix processed together (Chapter One). If samples are expected to contain target analytes of concern, laboratories may use one matrix spike and a duplicate of an unspiked field sample as an alternative to the MS/MSD pair (Sec. 9.4.3).

For samples requiring an extraction procedure separate from analysis (e.g., semivolatiles by Method 8270), the MS/MSD, or matrix spike and duplicate sample, should be extracted with the batch of samples but may be analyzed at any time. Conversely, if calibration standards and other analytical QC are processed identically to the field

samples (e.g., volatiles by Method 8260), the MS/MSD, or matrix spike and duplicate sample, should be prepared and analyzed concurrently with the samples.

When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. In the case of very contaminated samples or when the lab does not receive enough samples to perform a single matrix spike, an LCS and LCS duplicate (LCSD) may be performed to document precision and bias. An LCS should be included with each preparation batch. The LCS is an aliquot of the same clean (control) matrix used for the method blank(s) and of a similar weight or volume as the method blank and field samples. The LCS is spiked with similar analytes at the same concentrations as in the matrix spike and is processed identically to the samples.

In the case of samples that need an extraction procedure separate from analysis (e.g., semivolatiles by Method 8270), the LCS should be extracted with the batch of samples but may be analyzed at any time. However, if calibration standards and other analytical QC are processed identically to the field samples (e.g., volatiles by Method 8260), the LCS should be prepared and analyzed concurrently with the samples and may also serve as the continuing calibration verification (CCV) standard.

NOTE: If an LCS also serves as a CCV, acceptance criteria of the CCV should be used.

9.4.2 Spiking procedure for the MS/MSD and LCS

The solution used to fortify a sample and/or an LCS should contain all of the target analytes and their concentration levels should be determined as described in Secs. 9.4.1 and 9.4.2. For those methods that apply to a large list of analytes or that contain compounds that may interfere with an accurate assessment (i.e., coeluting or multi-peak analytes), a smaller subset of analytes may be used (see the specifics directions in the appropriate individual method).

9.4.2.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit or action level, the spike should be at or below the limit, or 1 - 5 times the background concentration (if historical data are available), whichever concentration is higher.

If historical data are not available, a background sample of the same matrix from the site may be submitted for matrix spiking purposes to ensure that high concentrations of target analytes and/or interferences will not prevent calculation of recoveries.

NOTE: If the background sample concentration is very low or non-detect, a spike of greater than 5 times the background concentration is still acceptable. To assess data precision with duplicate analyses, it is preferable to use a low concentration field sample to prepare a MS/MSD for organic analyses.

9.4.2.2 If the concentration of a specific analyte in a sample is not being checked against a limit specific to that analyte, then the analyst may spike the matrix spike or MS/MSD sample(s) at the same concentration as the reference sample (Sec. 9.3.3) at 20 times the estimated LLOQ in the matrix of interest, or at a concentration near the middle of the calibration range. It is suggested that a background sample of the same matrix from the site be submitted as a sample for matrix spiking purposes.

NOTE: Preparing the spiking solution from the same source as the calibration standards helps minimize additional variability due to differences between sources.

9.4.2.3 To develop precision and bias data for the spiked compounds, the analyst has two choices: analyze the original sample, and an MS/MSD pair; or analyze the original sample, a duplicate sample, and one spiked sample. If samples are not expected to contain the target analytes of concern, then the laboratory may use a MS/MSD pair. If samples are expected to contain the target analytes of concern, then the laboratory may use one matrix spike and a duplicate analysis of an unspiked field sample as an alternative to the MS/MSD pair.

9.4.2.4 Begin by analyzing one sample aliquot to determine the background concentration of each analyte. Prepare a matrix spike concentrate according to one of the options described in Sec. 9.4.2.1 or 9.4.2.2.

9.4.2.5 Prepare a matrix spike sample by adding the appropriate volume of the matrix spike concentrate to another aliquot of the sample to yield the desired concentration (Secs. 9.4.2.1 and 9.4.2.2). If MS/MSD analysis will be performed, prepare a matrix spike duplicate sample from a third aliquot of the sample.

9.4.2.6 Analyze the MS/MSD samples using the same procedures employed for the original sample and calculate the concentration of each analyte in the matrix spike and matrix spike duplicate. Likewise, analyze the LCS samples using the same procedures employed for the original sample, and calculate the concentration of each analyte in the LCS.

9.4.3 MS/MSD, Duplicate and LCS calculations

9.4.3.1 Calculation of % recovery (bias)

Bias is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the matrix spike, matrix spike duplicate (if performed) and LCS according to the following formula.

$$Recovery = \%R = \frac{(C_s - C_u)}{C_n}\ x\ 100$$

where:

C_s = Measured concentration of spiked sample aliquot

C_u = Measured concentration of unspiked sample aliquot (use 0 for LCS)

C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

MS/MSD recoveries may not be meaningful if the amount of analyte in the sample is large relative to the amount spiked.

9.4.3.2 Calculation of relative percent difference (RPD)

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} X\ 100$$

Precision is estimated from the RPD of the concentrations (not the recoveries) measured for MS/MSD pairs, or for duplicate analyses of unspiked samples. Calculate RPD according to the formula below.

where:

C_1 = Measured concentration of first sample aliquot

C_2 = Measured concentration of second sample aliquot.

NOTE: A difference in the amount of sample used for the MS/MSD results in an artificially high RPD when based on concentration. Using approximately the same sample size or scaling the spike amount to the sample size for the MS/MSD will minimize bias in the RPD calculation for MS/MSD.

9.4.4 Recommended QC acceptance criteria for matrix spike samples and LCS

The laboratory should develop performance data for precision and bias in the matrices of interest (Sec. 9.6). In addition, laboratories should monitor method performance in each matrix, through the use of control charts and other techniques.

Many methods may not contain recommended acceptance criteria for LCS results. The laboratory should use 70 - 130% as interim acceptance criteria for recoveries of spiked analytes, until in-house LCS limits are developed (Sec. 9.6). Where in-house limits have been developed for matrix spike percent recoveries, the LCS results should be similar to or tighter than those limits, as the LCS is prepared in a clean matrix.

Ideally, the acceptance criteria for MS/MSD recovery and/or duplicate relative % difference will be established for the field samples through the DQOs contained in a written QAPP. These criteria should be established with consideration given to performance data provided in the reference method and/or by the laboratory in order to avoid overly conservative expectations. In the absence of site- or project-specific acceptance criteria for matrix spike and duplicate QC samples, these criteria should be based on in-house performance data generated by the laboratory or on the performance data in the reference method.

Even when the project QAPP or determinative methods provide performance criteria for matrix spikes and LCS, laboratories must develop in-house performance criteria based on their historical data for use in project planning and for comparison to any relevant performance criteria in the reference methods. Development of in-house performance criteria is discussed in Sec. 9.6. Where methods do contain performance data for the matrix of interest, use Secs. 9.4.4.1 - 9.4.4.3 below as guidance in evaluating data generated by the laboratory.

9.4.4.1 When multi-laboratory performance data for the matrix of interest are provided in the determinative method, compare the recovery for each analyte with the method performance data of the same matrix. Given that such method performance criteria were developed from multi-laboratory data, they should be met by almost all laboratories. See Sec. 9.6.10 for more information on comparisons between limits. Performance data include an allowance for error in measurement of both the background and spike concentrations. If spiking was performed at a concentration substantially lower than the level used to generate the recovery data in the reference method, the recovery data in the method may not be appropriate for assessing the quality of the sample results, and criteria generated from in-house data may be more relevant.

9.4.4.2 When a method is initially established in a laboratory, the LCS limits may be applied to the matrix spikes until the laboratory has sufficient data (a minimum of 20 or more MS/MSD samples of the same matrix) to generate their own statistical limits. These data should be used as the basis for determining MS/MSD precision and bias limits. Alternatively, acceptance criteria based on historical LCS data may continue to be used for evaluating bias in matrix spike recovery and may be more sensitive to matrix effects than acceptance limits based on MS/MSD data. It is generally preferable to use statistically calculated MS/MSD, rather than LCS recovery limits once sufficient data points have been collected (i.e., ≥ 20 MS/MSD samples). See Secs. 9.6.1 - 9.6.3 for calculating in-house performance criteria for LCS, MS/MSD and surrogate recoveries.

9.4.5 Also, compare the recovery data from the matrix spike with the LCS data (use the average recovery if an MS/MSD were analyzed). If any individual %R in the MS/MSD falls outside the designated range for recovery, the laboratory should determine if there is a matrix effect or a laboratory performance problem. A matrix effect is indicated if the LCS data are within limits but the MS/MSD data exceed the limits. Surrogate recovery data (Sec. 9.5) should also be used to evaluate the data. Recoveries of both matrix spike compounds and surrogates outside of the acceptance limits suggest more pervasive analytical problems in the batch and/or instrument than problems with the recoveries of either matrix spikes or surrogates alone.

9.5 Surrogate recoveries

9.5.1 It is necessary that the laboratory evaluate surrogate recovery data from individual samples versus in-house surrogate recovery limits. General considerations for developing in-house acceptance criteria for surrogate recoveries are described in Sec. 9.6.

9.5.2 Surrogate recovery is calculated below.

$$Recovery\ (\%) = \left(\frac{Concentration\ (or\ amount)\ found}{Concentration\ (or\ amount)\ added}\right) \times 100$$

If recovery is not within in-house surrogate recovery limits, the following procedures are necessary.

9.5.2.1 Check for errors in the calculations, surrogate solutions or internal standards. If errors are found, recalculate the data accordingly.

9.5.2.2 Examine chromatograms for interfering peaks and proper peak integration.

9.5.2.3 Check instrument performance. If an instrument problem is identified, correct the problem and reanalyze the sample or extract.

9.5.2.4 If no instrument problem is found, the sample should be re-extracted and reanalyzed (or another vial reanalyzed for volatiles).

9.5.2.5 If upon re-analysis (in either 9.6.2.2 or 9.6.2.4), the recovery is again not within limits, report the data as an "estimated concentration." If the recovery is within the limits in the reanalysis, report the reanalysis data for the samples. If the method holding time for the sample has expired prior to re-extraction and/or reanalysis, qualify the data accordingly.

9.5.2.6 Some samples may need dilution to bring one or more target analytes within the calibration range or to overcome significant interferences. This may result in dilution of the surrogate responses to the point that recoveries cannot be measured. If surrogate recoveries are available from a less-diluted (or undiluted) aliquot of the sample or sample extract, those recoveries may be used to demonstrate that the surrogates are within the QC limits and no further action is needed.

9.6 Generation of performance criteria for MS/MSD, duplicates, surrogates, LCS, and IDP

Usefulness of developing in-house performance criteria and control charting or similar procedures to track laboratory performance cannot be overemphasized. Many data systems and commercially available software packages support the use of control charts.

Procedures for calculating in-house performance criteria for MS/MSD, LCS and surrogate recoveries are provided below.

9.6.1 Once sufficient data have been acquired and the recovery and RPD calculated as in Secs. 9.4.3 and 9.5 for a given sample matrix, the following statistics should be used to calculate acceptance criteria.

9.6.1.1 Mean percent recovery ($\bar{x}$) and standard deviation (s) for:

1) Each added target compound in the MS/MSD samples;
2) Each added target compound in the LCS samples; and
3) Each added surrogate in the field samples.

9.6.1.2 Mean RPD and standard deviation for MS/MSD or duplicate QC samples.

A minimum of 20 data points should be used to generate meaningful criteria. Inclusion of additional data should result in more robust criteria that better describe variance in method performance and result in fewer outliers. If the lower limit of the acceptance range is calculated to be <10%, it should be set to 10%. However, an alternative lower acceptance limit may be established by the laboratory or at the project level through the DQOs in a QAPP.

9.6.2 Calculate the upper and lower control limits for % recovery of each target or surrogate compound in LCS, MS/MSD and field samples using the respective $\bar{x}$ and *s* values calculated in Sec. 9.6.1.

Acceptance range = $\bar{x}$ (mean percent recovery) ± *3s* (standard deviation)

Upper control limit = $\bar{x}$ + *3s*
Lower control limit = $\bar{x}$ - *3s*

9.6.3 Calculate the upper control limit for the RPD for the MS/MSD using the mean RPD value + *3s* of the RPDs of historical MS/MSD pairs. RPD should be calculated based on the concentration or amount, not the spike recovery.

NOTE: The RPD limit only has a maximum value, because perfect agreement between C1 and C2 would result in a RPD of 0. Refer to Sec. 9.4.3.2 for the calculation.

9.6.4 Any matrix spike or surrogate recovery outside of control limits necessitates evaluation by the laboratory such as comparison with the LCS recovery.

9.6.4.1 If recoveries of analytes in the LCS are outside of the control limits, then the problem may lie with application of the extraction and/or cleanup procedures applied to the sample matrix, or with analysis. Once the problem has been identified and addressed, corrective action may include reanalysis of samples, or extraction and analysis of new sample aliquots, including new matrix spike samples and LCS. However, when there are a large number of analytes in the LCS or matrix spike, the statistical probability of a few analytes outside of control limits becomes high. Therefore, a number of analytes should be allowed to marginally fail the limits without requirement for corrective action. Laboratories should have a documented procedure to assess and qualify marginal exceedance limits.

9.6.4.2 When LCS results are within control limits but matrix spike results are not, the problem may either be related to the specific sample matrix or to an inappropriate choice of extraction, cleanup, and/or determinative method. If results are to be used for regulatory compliance monitoring, the analyst must take steps to demonstrate that the analytes of concern can be determined in the sample matrix at the levels of interest.

9.6.5 Once established, control limits should be reviewed regularly and updated on a routine basis as established by the laboratory's quality management plan. Reviewing appropriateness of these criteria with respect to generated data is especially important for newly implemented procedures or those not in continual use. The laboratory should monitor trends in both analyte recovery performance and also in the control limits. Control limits used to evaluate sample results should be those in place at the time of sample analysis. Once control limits are updated, they should apply to all subsequent analyses of new samples.

9.6.6 For analytes, methods, and matrices with very limited data (e.g., unusual analytes or matrices not analyzed often), interim limits should be established using available data or by analogy to similar methods or matrices.

9.6.7 Results used to develop acceptance criteria should meet all other QC criteria associated with the determinative method. For example, matrix spike recoveries

from a GC/MS procedure should be generated from samples analyzed after a valid tune and initial calibration that includes the matrix spike compounds. Analytes in GC or HPLC methods should fall within the established retention time windows in order to be used to develop acceptance criteria.

9.6.8 Laboratories are advised to consider effects of spiking concentration on matrix spike performance criteria. Acceptance criteria for matrix spike recovery and precision are often a function of the spike concentration used. Therefore, use caution when pooling data in establishing acceptance criteria. Not only should results all be from roughly the same type matrix but spiking levels should also be similar.

9.6.9 Similarly, acceptance criteria for matrix spike, LCS and surrogate results should all be generated using the same combination of extraction, cleanup, and analysis techniques. For example, do not mix results from solid samples extracted by ultrasonic extraction with those extracted by Soxhlet.

9.6.10 Another common error in developing acceptance criteria is discarding data that do not meet a preconceived notion of acceptable performance (i.e., while professional judgment is important in evaluating data used to develop acceptance criteria, do not discard specific results simply because they do not meet one's expectations). This practice results in a censored data set, which when used to develop acceptance criteria, will lead to unrealistically narrow criteria. Rather, employ a statistical test for outlier values, or at least calculate the acceptance limits both with and without the results considered suspect. Then, observe the effect of deleting suspect data.

9.6.11 In-house QC limits must be examined for reasonableness; it is not EPA's intent to legitimize poor recoveries due to incorrect choice of methods or spiking levels.

In-house limits should also be compared with the DQOs of specific analyses. For example, recovery limits (for surrogates, MS/MSD, LCS, etc.) that include allowance for a relatively high positive bias (e.g., 70 - 170%) may be appropriate for determining that an analyte is <u>not</u> present in a sample. However, they would be less appropriate for analysis of samples near but below a regulatory limit because of the potential for high bias.

9.6.12 It may be useful to compare QC limits generated in the laboratory with performance data listed in specific determinative methods. However, the analyst must be aware that performance data generated from multi-laboratory studies tend to be significantly wider than those generated from a single laboratory.

9.7 Lower Limit of Quantitation (LLOQ)

The LLOQ is the lowest concentration at which the laboratory has demonstrated target analytes can be reliably measured and reported with a certain degree of confidence, which must be ≥ the lowest point in the calibration curve. The laboratory shall establish the LLOQ at concentrations where both quantitative and qualitative requirements can consistently be met (see Sections 9.7.3 and 11.6). The laboratory shall verify the LLOQ at least annually, and whenever significant changes are made to the preparation and/or analytical procedure, to demonstrate quantitation capability at lower analyte concentration levels. The verification is performed by the extraction and/or analysis of an LCS (or matrix spike) at 0.5-2 times the established LLOQ. Additional LLOQ verifications may be useful on a project-specific basis if a matrix is expected to contain significant interferences at the LLOQ. The verification may be accomplished with either clean control material (e.g., reagent water, solvent blank, Ottawa sand, diatomaceous earth) or a representative sample matrix, free of target compounds. Optimally,

the LLOQ should be less than the desired decision level or regulatory action level based on the stated DQOs.

9.7.1 LLOQ Verification – The verification of LLOQs using spiked clean control material represents a best-case scenario because it does not evaluate the potential matrix effects of real-world samples. For the application of LLOQs on a project-specific basis, with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.7.2 The LLOQ verification (to be performed after the initial calibration) is prepared by spiking a clean control material with the analyte(s) of interest at 0.5-2 times the LLOQ concentration level(s). Alternatively, a representative sample matrix free of targets may be spiked with the analytes of interest at 0.5-2 times the LLOQ concentration levels. The LLOQ check is carried through the same preparation and analytical procedures as environmental samples and other QC samples. It is recommended to analyze the LLOQ verification on every instrument where data is reported; however, at a minimum, the lab should rotate the verification among similar analytical instruments such that all are included within 3 years. Frequently performed analyses, such as Methods 8260C and 8270D, should have an LLOQ check standard be verified, at minimum, once a year.

9.7.3 Recovery of target analytes in the LLOQ verification should be within established in-house limits or within other such project-specific acceptance limits to demonstrate acceptable method performance at the LLOQ. Until the laboratory has sufficient data to determine acceptance limits, the LCS criteria ± 20% (i.e., lower limit minus 20% and upper limit plus 20%) may be used for the LLOQ acceptance criteria. This practice acknowledges the potential for greater uncertainty at the low end of the calibration curve. Where practical, historically based LLOQ acceptance criteria should be determined once sufficient data points have been acquired.

9.7.4 Reporting concentrations below LLOQ - Concentrations that are below the established LLOQ may still be reported; however, these analytes must be qualified as estimated. The procedure for reporting analytes below the LLOQ should be documented in the laboratory's SOP or in a project-specific plan. Analytes below the LLOQ that are reported should meet most or all of the qualitative identification requirements in Sec. 11.6.

9.8 It is recommended that the laboratory adopt additional QA practices for use with 8000-series methods. Specific practices that are most productive depend upon the needs of the laboratory, nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess precision of the environmental measurements. When doubt exists over identification of a peak on the chromatogram, confirmatory techniques such as GC with a dissimilar column, element-specific detector, or mass spectrometer (selected ion monitoring or full scan) must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

Refer to the appropriate determinative method for detailed calibration and standardization procedures and the general guidance as noted in Sec. 11.0.

11.0 PROCEDURE

Extraction and cleanup are critical for successful analysis of environmental samples and wastes. Analysts should pay particular attention to selection of sample preparation procedures to obtain reliable measurements.

11.1 Extraction

Individual determinative methods for organic analytes in SW-846 often recommend appropriate sample extraction procedures. General guidance on semivolatile extraction procedures can be found in Method 3500. Guidance on volatile procedures can be found in Method 5000.

11.2 Cleanup and separation

Individual determinative methods for organic analytes in SW-846 often recommend appropriate cleanup procedures. General guidance on cleanup procedures can be found in Method 3600. While some relatively clean matrices (e.g., groundwater samples) may not need extensive cleanups, the analyst should carefully balance time savings gained by skipping cleanups against potential increases in instrument downtime and loss of data quality that can occur as a result.

11.3 Chromatographic Performance

Recommended chromatographic columns and instrument conditions are described in each determinative method. As noted earlier, these columns and conditions are typically those used during development and testing of the method. However, other chromatographic systems may have somewhat different characteristics as analytical instrumentation continues to evolve. Therefore, SW-846 methods allow analysts flexibility to change conditions as long as adequate performance is demonstrated.

Chromatographic performance is demonstrated by resolution of standards and ability to model the response of the detector during calibration; also important are sensitivity, bias, precision, frequency of false positives, and frequency of false negatives during analysis. For any chromatographic procedures or conditions used, the laboratory must demonstrate that the performance satisfies the analytical requirements of the specific application for which the chromatographic procedure is being used. Such demonstrations should be performed using procedures outlined in Secs. 9.2 - 9.5 of this method and in Chapter One.

11.4 Initial Calibration

Calibration of an analytical instrument involves delineation of the relationship between response of the instrument and amount or concentration of an introduced analyte. The graphical depiction of this relationship is often referred to as the calibration curve. To perform quantitative measurements, this relationship, termed initial calibration, must be established before the analyses of any samples.

Historically, many analytical methods have relied on linear models of the calibration relationship, where the instrument response is directly proportional to the amount of a target compound. The linear model has many advantages including simplicity and ease of use. However, given the advent of new detection techniques and because many methods cannot be optimized for all the analytes to which they may be applied, the analyst is increasingly likely to encounter situations where the linear model neither applies nor is appropriate.

Initial calibration for SW-846 chromatographic methods involves analysis of standards containing the target compounds at a minimum of five different concentrations within the working range of the instrument. In order to produce acceptable sample results, instrument response must be within the range established by the initial calibration.

Extrapolation of the calibration to concentrations above or below those of the actual calibration standards is not appropriate and may lead to significant quantitative errors, regardless of the calibration model chosen. It may be necessary to prepare calibration standards that cover concentration ranges appropriate for specific projects or types of analyses. For instance, the analyst should not necessarily expect to perform a calibration appropriate for sub-ppb level analyses and use the same calibration data for high-ppb or ppm-level samples. Preparation of calibration standards is described in general terms in Sec. 11.4.1.

SW-846 methods in this manual for quantitative chromatographic analysis rely on one of three commonly used calibration approaches:

- External standard calibration
- Internal standard calibration
- Isotope dilution calibration

These approaches are described in general terms in Secs. 11.4.2 - 11.4.4.

General calibration criteria are provided in Sec. 11.5 for GC and HPLC procedures using non-MS detection. Calibration procedures for GC/MS (e.g., Methods 8260, 8270, 8276, 8280, and 8290), HPLC/MS (e.g., Methods 8321 and 8325), and GC/FT-IR (e.g., Method 8410) are described in those methods. Some determinative methods may provide specific guidance on calibration such as Method 8085, GC/AED with compound-independent calibration.

Regardless of the specific calibration technique used, introduce calibration standards and samples into the instrument by the same technique and at the same volume. Tabulate peak responses against the mass or concentration introduced as described in Secs. 11.4.2 - 11.4.4.

11.4.1 Preparation of calibration standards

Calibration standards are prepared using specific procedures indicated in the determinative methods. However, the general procedure is described here.

11.4.1.1 For each analyte and surrogate of interest, prepare calibration standards at a minimum of five different concentrations with an appropriate solvent. Alternatively, provided they meet the objectives of the intended application, prepared standards may be purchased from commercial suppliers.

NOTE: As previously mentioned in Sec. 1.9, it may not be practical or appropriate to attempt to determine simultaneously all analytes listed in a given method. Analyte lists in the determinative methods do not imply a regulatory requirement for analysis of any or all of the compounds but rather indicate the applicable method(s). Therefore, if an analyte is not relevant to a specific project, it need not be included in the calibration standards associated with that project.

11.4.1.2 The lowest concentration calibration standard analyzed during an initial calibration will generally establish the LLOQ. (See Sec. 9.7 for

suggested LLOQ establishment and verification.) The analyte concentration in the lowest standard is related back to a sample concentration using the sample amount, dilution, and final injection volume used for the specific analysis. Thus, changes to specific sample amounts, dilutions, and volumes employed will be reflected in the LLOQs for samples.

11.4.1.3 Higher concentrations should define the working range of the detector or correspond to the expected range of concentrations found in samples that are also within the working range of the detector. Standards prepared by serial dilution of a stock solution will typically form a geometric series in which the concentrations or amounts of each standard vary from the adjacent standards by a constant factor, e.g., 10, 20, 40, 80, and 160 ng.

However, the relatively wide spacing of upper standards in a geometric series can mask the situation where a detector is reaching saturation and the instrument responses are leveling off somewhere between the last two standards. Therefore, it may be preferable to use a partial arithmetic series in which concentrations of the upper standards differ by a constant amount, not a constant factor. Using the same overall calibration range as in the example above, one such series might be 10, 20, 40, 80, 120, and 160 ng, with a constant difference of 40 ng between the top four standards and resulting in a six-point calibration that will better define the instrument response.

NOTE: Amounts shown above are for illustrative purposes only. Both the overall calibration range and concentrations or amounts used for the standards are a function of the specific instrumentation, demonstrable working range of instrumentation, and intended application of the specific method. Therefore, laboratories must determine the calibration range and standards for their specific circumstances.

11.4.1.4 For each analyte, at least one of the calibration standards MUST correspond to a sample concentration at or below the quantitation levels needed for the project; this may include establishing compliance with a regulatory or action limit. Given that different limits may be associated with different analytes, the same standard should not be expected to fulfill this requirement for all analytes.

11.4.1.5 Given the large number of target compounds addressed by some of the methods listed in Sec. 1.1, it may be necessary to prepare several sets of calibration standards, each set consisting of different analytes. Initial calibration will then involve analysis of each of these sets of standards.

11.4.2 External standard calibration

External standard calibration is one of the most common approaches to calibrations. It involves a simple comparison of instrument responses from the sample to the target compound responses in the calibration standards. Sample peak responses are compared with calibration standard peak responses. The ratio of the detector response to the amount (mass) of analyte in the calibration standard is defined as the calibration factor (CF).

$$\mathrm{CF} = \frac{peak\ response\ of\ the\ standard\ compound}{mass\ of\ the\ compound\ (nanograms)}$$

Advantages of external standard calibration are that it is simple and can be applied to a wide variety of specific chromatographic methods. The primary disadvantage is that it is greatly affected by stability of the chromatographic detector system and presence of chromatographic interferences in a sample or sample extract.

The CF may also be calculated using the standard concentration rather than mass in the denominator of the equation above. However, use of concentration in calculating the CF will necessitate changes to the equations used to calculate sample concentration (Sec. 11.10.3).

For multi-component analytes (e.g., PCBs and toxaphene), see the appropriate determinative method for information on which peaks to employ for CF calculation.

11.4.3 Internal standard calibration

Internal standard calibration involves comparison of instrument responses from the target compounds in the sample to responses of other standards added to the sample or extract before injection. Response of the target compound is normalized to the response of the other standard. This other standard is called an internal standard because it is contained within the aliquot of the sample or sample extract injected into the instrumentation.

A constant amount of the internal standard is added to all samples or extracts. That same amount of the internal standard is also included in each of the calibration standards. In the sample or sample extract, the peak response ratio of the target compound to the internal standard is compared with a similar ratio derived for each calibration standard. This ratio is termed the response factor (RF) or relative response factor (RRF), indicating that the target compound response is calculated relative to that of the internal standard.

Advantages of internal standard calibration include that it can account for routine change in response of the chromatographic system as well as variation in the volume of the introduced sample or sample extract. In addition to normalizing the peak response of the target compound to the response of the internal standard in the sample or extract for that injection, the internal standard may be used to calculate the relative retention time (RRT) of the target compound. RRT is expressed as a unitless quantity.

$$RRT = \frac{Retention\ time\ of\ the\ analyte}{Retention\ time\ of\ the\ internal\ standard}$$

If RRT is used by the laboratory to establish peak identity, the RRT of each target analyte in each calibration standard should agree within established limits set by the laboratory in their SOP. These limits should be appropriate for reliable identification of the target analyte. If this criterion is not met and there are no other indicators of an analyte's identification (such as a very unique and high probability mass spectral match), that analyte may not be considered as identified by RRT.

The RRT of the analyte in the sample should be within the RRT limits of the analyte in the standards. If this criterion is not met and there are no other indicators of a component's identification (such as a very unique and high probability mass spectral match), that component may not be considered as identified by RRT.

RRT evaluation allows the analyst to compensate for modest shifts in the chromatographic conditions that can occur due to interferences and day-to-day instrument variability. Many methods that employ internal standard calibration use more than one internal standard; target compounds are related to the internal standards based on similarity of their respective chromatographic retention times or physical and chemical properties.

Principal disadvantages of internal standard calibration are that internal standards must be compounds not found in the samples to be analyzed and they must produce an unambiguous response on the chromatographic detector system. Many SW-846 methods recommend brominated or fluorinated compounds and/or stable isotopically labeled analogs of target compounds (e.g., a compound containing a deuterium atom instead of hydrogen, or a ^{13}C atom instead of a ^{12}C atom) as internal standards. Isotopically labeled compounds are most often employed in MS detection methods because the detector can differentiate between the target compound and the labeled internal standard based on its added mass even when the two compounds elute at the same retention time. In general, internal standard calibration is not as useful for GC and HPLC methods with non-MS detectors because of the inability to chromatographically resolve many internal standards from the target compounds.

Internal standards recommended in many SW-846 methods were used during development of the method. Analysts may employ other internal standards in place of, or in addition to, those recommended. If internal standards are not recommended in the method, the analyst should select one or more compounds similar in analytical behavior to the analytes of interest and not expected to be found in the samples. Whichever internal standards are employed, the analyst should demonstrate (as detailed in the determinative method) that measurement of the internal standard is not affected by target analytes, surrogates, or matrix interferences.

When preparing calibration standards, add the same amount of the internal standard solution to each calibration standard. Therefore, the internal standard concentration is the same in each calibration standard, whereas concentrations of the target analytes will vary. The internal standard solution may contain more than one internal standard, and their relative concentrations may differ within the spiking solution. However, the mass of each internal standard added to the samples or sample extracts immediately before injection must be the same as in each calibration standard. The volume of the internal standard solution spiked into a sample extract should cause minimal dilution of the extract.

An ideal internal standard concentration would yield a RF of 1 for each analyte. However, this is unlikely to be the case when dealing with more than a few target analytes. Therefore, as a general rule, the internal standard should produce an instrument response ≤ 100 times that produced by the least responsive target analyte associated with the internal standard. This should result in a minimum RF of approximately 0.01 for the least responsive target compound.

For each of the initial calibration standards, calculate the RF values for each target analyte relative to one of the internal standards as follows.

$$RF = \frac{A_s \, x \, C_{is}}{A_{is} \, x \, C_s}$$

where:

A_s =Peak response of the analyte or surrogate
A_{is}=Peak response of the internal standard
C_s =Mass of the analyte or surrogate in the sample aliquot
C_{is}=Mass of the internal standard in the sample aliquot

Response factors for GC/MS methods may also be calculated using sums of the areas of two ions (expressed as mass over charge, *m/z*) for each target analyte and internal standard.

Note that in the equation above, RF is unitless. Therefore, units such as ng or µg may be used for amounts of the analyte, surrogate, and internal standard, provided that they are uniform.

Because internal standards are used to compensate for routine variations in the chromatographic separation of target compounds, there is a significant advantage to using more than one internal standard when dealing with a large number of target compounds or when those compounds elute over a long timeframe. When multiple internal standards are employed, target compounds are associated with the internal standards on the basis of their respective retention times. Therefore, the internal standards should be chosen to cover the expected retention time range of the target compounds. Accordingly, internal standards can compensate for small retention time shifts or response changes in the portion of the chromatographic run in which they occur. Ideally, the analyst will employ enough internal standards to result in a RRT for each target compound in the range 0.80 - 1.20, though other RRT ranges may be appropriate as well.

Many methods that utilize internal standard calibration include acceptance limits for responses of the internal standards in the calibration standards, samples, or both. Those limits are typically expressed in terms of peak areas because the concentration of the internal standard cannot be measured directly (e.g., one has to assume that the entire mass injected into the sample or sample extract is present during analysis). Common consensus limits are 50 - 200% of the area of the internal standard in the most recent calibration standard. Representing a factor of two, these limits are used as a gross diagnostic check on addition of the internal standards to the samples or extracts and injection of the sample aliquot into the instrument.

11.4.4 Isotope dilution calibration

Isotope dilution calibration is a special case of internal standard calibration. In isotope dilution, the internal standards are stable isotopically labeled analogs of the target analytes and are added to the sample prior to extraction or other sample preparation steps such as pH adjustment, drying, or extraction solvent addition. Physical and chemical properties of each labeled compound are virtually the same as its unlabeled "native" analog. Thus, any losses of the target compound that may occur during sample preparation or determinative steps will be mirrored by a similar loss of the labeled standard. Similarities between labeled compounds and unlabeled analogs mean that RFs and RRTs for the unlabeled compounds are very close to 1.0.

Labeled compounds are spiked into samples and standards at a constant amount. RFs developed from the calibration standards assume that all of the labeled compounds added to the sample reach the instrument. This assumption, termed recovery correction, allows for correction to observed concentrations of the target compound relative to its labeled counterpart.

The degree to which the labeled compounds meet this assumption is monitored by use of traditional internal standards added to the sample extract immediately prior to injection. Separate RFs relate the concentrations of the labeled compounds to the traditional internal standards. Most isotope dilution methods include some limits on the apparent recovery of the labeled compounds. However, those limits are often consensus limits that may be overly conservative. As long as responses for both native and labeled compounds can be distinguished from the background instrumental noise, isotope dilution calibration can provide excellent results, even when the apparent spike recovery of the labeled compound is low. Labeled compound recoveries >100% are allowed as well. Such recoveries can occur as a result of the inherent variability in calibration of the labeled compounds and are not indicative of contamination or other problems.

Built-in recovery correction is one of the principal advantages of isotope dilution calibration. Isotope dilution requires an MS detection system and isotopically labeled analogs of target analytes and generally produces more precise data with lower bias. The added cost of isotopically labeled compounds is a disadvantage, but can be offset by higher quality data, as well as eliminating some routine QC analyses, such as surrogates and MS/MSDs used with internal standard calibration. However, whether or not to add surrogate or prepare MS/MSD aliquots should be described in a QAPP and not left to the analyst's professional judgment.

Isotope dilution calibration is often used in conjunction with selected ion monitoring (SIM) GC/MS procedures, such as those for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. These procedures, along with a relatively small list of target compounds, allow the instrument to be operated in a mode that detects only those ions (*m/z*) corresponding to the target compounds and their labeled analogs; this application significantly increases the sensitivity of the method and reduces interferences. Because isotope dilution methods have acceptance limits for recovery of the labeled analogs added prior to extraction or other sample preparation steps, these methods typically do not contain limits on responses of the traditional internal standards used to monitor those recoveries.

A RF is calculated for each target compound relative to its labeled analog and for each labeled analog relative to the traditional internal standard added immediately prior to injection. These calculations may involve areas of more than one ion (*m/z*) for each compound (e.g., Methods 8280 and 8290).

11.4.5 Extracted internal standards

Extracted internal standard calibration is a hybrid of internal standard calibration and isotope dilution calibration. In this approach, traditional internal standards are added to the samples before extraction instead of just prior to analysis. Results for the target compounds can be corrected for recovery of the internal standards using the same assumptions made for isotope dilution.

This approach is most helpful when the compounds used as internal standards are very closely related to the target compounds. It is similar to the internal standard procedure used for volatile organic analyses by purge-and-trap or headspace extraction.

11.5 Calibration models and acceptance criteria

SW-846 chromatographic methods allow the use of three different calibration models: average calibration factor or response factor (Sec. 11.5.1), linear regression (Sec. 11.5.2), and non-linear regression (Sec. 11.5.3). Any of these models can be applied to either external or internal standard calibration data. This section also provides suggested criteria for calibration models; however, method- or project-specific criteria will always supersede general guidance.

Choice of calibration model may begin with the simplest approach, the average calibration factor or response factor model, and then progress through linear and then non-linear regression until the calibration acceptance criteria are met. Another appropriate approach is to choose a calibration model based on previous experience, knowledge of the physics of the detector, or specific manufacturer's recommendations. For the calibration model to be usable, it must be continuous and monotonic throughout the calibration range. More calibration points are required for more complex models. The chromatographic methods in SW-846 employ a minimum of five standards for average response factor or linear (first-order) calibration models, six standards for a quadratic (second-order) model, and seven standards for a cubic (third-order) model.

These calibration models and calculations may be applied to any sort of chromatographic instruments in use, such as, but not limited to: GC, GC/MS, liquid chromatographs (LC), LC/MS or HPLC. They may also be applied to any instruments using various types of detectors, including anything from traditional GC detector types (FID, ELCD, ECD, NPD, diode array, UV, visible light wavelengths, and MSs (whether single, MS/MS, ion trap or time-of-flight).

NOTE: The option of using non-linear calibration may be necessary to address specific instrumental techniques. However, it is not EPA's intent to allow non-linear calibration to compensate for detector saturation or avoid proper instrument maintenance. Regardless of the calibration model chosen, an *X* value of zero should not be included as a calibration point.

The following sections describe various options for initial calibration evaluation and provide the calibration acceptance criteria used to evaluate each option. In addition to this suggested acceptance criteria, two general methods for assessing the accuracy of the calibration curve for all allowed curve models are presented in 11.5.4. It is further recommended that each calibration model be inspected to ensure that the data are representative of the model chosen as described in Sec. 11.5.6. Whichever calibration model is selected, samples with concentrations that exceed the calibration range must be diluted to fall within the range. Criteria listed in these sections are designed for quantitation of trace-level concentrations of the analytes of interest. If data of lower quality will satisfy project-specific data needs, less stringent criteria may be employed provided they are documented and approved in a QAPP.

11.5.1 Linear calibration using average calibration or response factor

As calculated in Sec 11.4, each CF or RF represents the slope of the line between the origin and the given standard response. If the relative standard deviation (RSD) of variation in the factors is ≤ 20%, the linear model is generally representative over the range of calibration standards. Representativeness beyond the range of calibration standards is not to be inferred. At least five calibration levels should be used to construct

the average CF or RF model (Sec. 11.5).

To evaluate linearity of the initial calibration, calculate the mean CF (external standard calibration) or RF (internal standard calibration), the standard deviation (SD), and the RSD (also called coefficient of variance, CV) as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}\left(CF_i - \overline{CF}\right)^2}{n-1}} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n}\left(RF_i - \overline{RF}\right)^2}{n-1}}$$

$$mean\ CF = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n} \qquad mean\ RF = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n}$$

$$RSD = \frac{SD}{\overline{CF}}\ x\ 100 \qquad RSD = \frac{SD}{\overline{RF}}\ x\ 100$$

Where *n* is the number of calibration standards and RSD is expressed as a percentage (%).

11.5.1.1 If the RSD is ≤20% over the calibration range, the slopes of the lines for each standard are sufficiently close to one another that the use of the linear model is generally appropriate over the range of standards that are analyzed; $\overline{CF}$ or $\overline{RF}$ may be used to determine sample concentrations. Alternatively, either of the two methods described in 11.5.4 may be used to determine calibration function acceptability.

NOTE: The RSD approach is equivalent to a $1/x^2$ weighted linear least square regression line that is forced through the origin.

11.5.1.2 Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed the acceptance limit for the RSD for a given calibration. In those instances, it is recommended, but not required, that corrective actions as described in Sec. 11.5.6.1 be followed. Sec. 11.5.6.1 also provides alternative uses for initial calibrations that do not meet their criteria of acceptability.

11.5.1.3 Calculation of sample amounts

If all the conditions in Secs. 11.5.1.1 and 11.5.1.2 are met, the $\overline{CF}$ or $\overline{RF}$ may be used to determine sample concentrations, as described in Sec. 11.10. It is recommended that the curve generated by the $\overline{CF}$ or $\overline{RF}$ be examined for acceptability using the refitting check described in Sec. 11.5.4.1. The calculated amount introduced into the instrument, x_s, is:

$$X_s = \frac{A_s}{\overline{CF}} \qquad and \qquad X_s = \frac{A_s}{\overline{RF}}\ x\ \frac{C_{is}}{A_{is}}$$

where:

X_s = Calculated mass of the analyte or surrogate in the sample aliquot introduced into the instrument (in ng)

A_s = Peak response of the analyte or surrogate in the sample

A_{is} = Peak response of the internal standard in the sample

C_{is} = Mass of the internal standard in the sample aliquot introduced into the instrument (in ng)

$\overline{CF}$ = Average calibration factor from the most recent initial calibration

$\overline{RF}$ = Average RF from the most recent initial calibration.

Units for the mass of analyte should be the same units used to calculate the CFs or RFs. If different units are used for amount (e.g., µg/L), these calculations and those found in Sec. 11.10 should be adjusted accordingly.

11.5.2 Linear calibration using a least squares regression

A linear calibration model based on a least squares regression may be employed based on past experience or a priori knowledge of the instrument response. Based on the professional judgment of the analyst, this approach also may be used for analytes that do meet the RSD criteria in Sec. 11.5.1. This is most easily achieved by performing a linear least squares regression of the instrument response versus the mass of the chromatographed analyte. Treat the instrument response as the dependent variable (*y*) and the amount as the independent variable (*x*). This is a statistical requirement and is not simply a graphical convention. At least five calibration levels should be used to construct the linear regression model (Sec. 11.5).

For external standard calibration, *x* is the mass of the analyte in the sample aliquot introduced into the instrument and *y* is the instrument response.

$$x = C_S \qquad\qquad y = A_S$$

For an internal standard calibration, x and *y* can be assigned in various ways where *x* is the amount of the analyte introduced into the instrument and *y* is the instrument response to that analyte. Two options are provided here using the mass introduced into the instrument. If other assignments for *x* and *y* are used, e.g., concentration, subsequent equations used for calculating mass of the analyte introduced into the instrument must be changed accordingly.

Option 1: X_s is the mass of the analyte in the calibration standard aliquot introduced into the instrument and Y_s is the ratio of response of the analyte to the response of internal standard times the mass of the internal standard in the calibration standard aliquot introduced into the instrument.

$$X_s = C_s \qquad and \qquad Y_s = A_s \text{ x } \frac{C_{is}}{A_{is}}$$

Option 2: *x* is the ratio of the analyte mass in the calibration standard aliquot introduced into the instrument to the internal standard mass in the calibration standard aliquot introduced into the instrument and *y* is the ratio of response of the analyte to the response of internal standard.

$$x = \frac{C_S}{C_{is}} \qquad\qquad y = \frac{A_S}{A_{is}}$$

where:

C_s = Mass of analyte in the volume of calibration standard introduced into the instrument.

C_{is} = Mass of internal standard in the volume of calibration standard injected into the instrument.

A_s = Peak response of analyte.

A_{is} = Peak response of internal standard.

A linear least squares regression attempts to construct a linear equation of the form:

$$y = ax + b$$

by minimizing the sum of squared differences between the observed response (y_i, the instrument response) and the predicted response (y'_i, the response calculated from the constructed equation) at each calibration level.

Weighting the sum of the squares of the differences may significantly improve the ability of the least squares regression to fit the linear model to the data, especially at the low end of the calibration curve. The general form of the sum of the squares of the differences containing the weighting factor is:

$$\sum_{i=1}^{n} W_i (y_i - y'_i)^2$$

where:

W_i = Weighting factor for the i^{th} calibration standard (w=1 for unweighted least squares regression, or 1/x or $1/x^2$ for weighted least squares regression)

y_i = Observed instrument response for the i^{th} calibration standard.

y'_i = Predicted (or calculated) response for the i^{th} calibration standard.

n = Total number of calibration standards.

Mathematics used in least squares regression favors numbers of larger value over numbers of smaller value. Thus, unweighted regression curves will tend to fit points that are at upper calibration levels better than those points at lower calibration levels. If concentrations of concern are at lower calibration levels, an unweighted regression curve tends to give less accurate results. A weighting factor which reduces this tendency can be used as compensation.

11.5.2.1 Do not include an X-value of zero as a calibration point. However, most data systems and many commercial software packages will allow the analyst to "force" the regression through the origin. Forcing the curve through the origin is not the same as including the origin as a fictitious point in the calibration. In essence, if the curve is forced through the origin, the intercept is set to 0 *before* the regression is calculated, thereby setting the bias to favor the low end of the calibration range by "pivoting" the function around the origin to find the best fit and resulting in one less degree of freedom. It may be appropriate to force an unweighted regression though the origin for some calibrations, but not when the regression is weighted.

However, forcing the regression through the origin may NOT be used as a rationale for reporting results below the calibration range demonstrated by the analysis of the standards. Results should not be reported at a concentration below the LLOQ unless qualified as estimated.

11.5.2.2 In the specific case of an unweighted linear least squares regression (i.e., a regression that varies both a and b), the correlation coefficient (*r*) can be used to measure the "goodness of fit."

$$r = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{\left(\sqrt{n\sum_{i=1}^{n} x_i{}^2 - \left(\sum_{i=1}^{n} x_i\right)^2}\right)\left(\sqrt{n\sum_{i=1}^{n} y_i{}^2 - \left(\sum_{i=1}^{n} y_i\right)^2}\right)}$$

The value of r is such that $-1 \leq r \leq +1$.

The instrument data system will typically calculate *r.* An *r*-value of +1.00 indicates a positive perfect correlation; an *r*-value of -1.00 indicates a negative perfect correlation; an *r*-value of 0 indicates no correlation.

However, if the regression line is forced through the origin or the weighting factor is variable, then the coefficient of determination, more often termed r^2, should be used to measure the "goodness of fit", such that $0 \leq r^2 \leq 1$. This shows the strength of the association between *x* and *y*. The r^2 value allows the analyst to determine the percent of the data closest to the line of best fit. For consistency, it is acceptable to use r^2 for linear unweighted curves as well. An r^2 value of 1.00 indicates that all variability in response is due to variation in concentration.

In order for the linear regression model to be used for quantitative purposes, *r* or r^2 should be ≥ 0.995 or 0.99, respectively. Alternatively, either of the two methods described in Sec. 11.5.4 may be used to determine whether the calibration function meets acceptance criteria. It is recommended that the resulting calibration curve be inspected by the analyst as described in Sec. 11.5.4.1.

11.5.2.3 To calculate the mass (*x*) of the analyte in the sample aliquot introduced into the instrument, the regression equation is rearranged.

$$x = \frac{(y - b)}{a}$$

External standard calibration allows the mass of the analyte in the sample aliquot introduced into the instrument to be calculated.

$$x_S = \frac{(A_S - b)}{a}$$

For the internal standard method, the calculation will depend on which of the two options described in Sec. 11.5.2 is chosen.

$$Option\ 1 \qquad X_s = \frac{\left(\frac{(A_s \text{ x } C_{is})}{A_{is}}\right) - b}{a}$$

$$Option\ 2 \qquad X_s = \frac{\left(\left(\frac{A_s}{A_{is}}\right) - \ b\ \right) \text{ x } C_{is}}{a}$$

where:

X_s = Calculated mass of the analyte or surrogate in the sample aliquot introduced into the instrument (in ng)
A_s = Peak response of the analyte or surrogate in the sample
A_{is} = Peak response of the internal standard in the sample
C_{is} = Mass of the internal standard in the sample aliquot introduced into the instrument (in ng).

Units for analyte mass should be the same as those used to determine the regression equation. If alternative units such as concentrations are used, calculations for the final sample concentrations found in Sec. 11.10 should be adjusted accordingly.

11.5.3 Non-linear calibration

In situations where the analyst knows the instrument response does not follow a linear model over a sufficiently wide calibration range, or when other approaches described here have not met acceptance criteria, a non-linear calibration model may be employed. At least six calibration levels are recommended to construct a quadratic (second-order) calibration curve, and at least seven levels should be used for a cubic (third-order) curve (Sec. 11.5).

NOTE: It is not EPA's intent to allow non-linear calibration to compensate for detector saturation or to avoid proper instrument maintenance.

When a calibration model for quantitation is used, the curve must be continuous: continuously differentiable and monotonic over the calibration range. The model chosen should have no more than four parameters, as in this equation:

$$y = f(a, b, c, d, x)$$

where *f* indicates a function with up to four parameters, *a* – *d*, and *x* is the independent variable. If the model is polynomial, it may be no more than third-order, as in the equation:

$$y = ax^3 + bx^2 + cx + d$$

When the linear regression model is used to estimate model parameters for the calibration data, the instrumental response (*y*) must be treated as the dependent variable, and the amount of the calibration standard (*x*) must be the independent variable. An *x*-value of zero should not be included as a calibration point, although the curve may either be weighted or forced through the origin as long as calibration criteria are met.

Model estimates from the regression must be used as calculated, and no term (i.e., *a, b, c, or d*) calculated as a result of the least squares regression can be modified. Weighting in a calibration model or forcing through the origin may significantly improve the ability of the least squares regression to fit the data at lower concentration levels. However, forcing the regression through the origin may NOT be used as a rationale for reporting results below the calibration range demonstrated by the analysis of the standards.

11.5.3.1 Linear and non-linear least squares regressions are mathematical methods that minimize differences (the residuals) between observed instrument response, y_i, and calculated response, y_i', by adjusting coefficients of the polynomial (*a, b, c, and d*) to obtain the polynomial best fitting the data.

The coefficient of determination (r^2) may be used as a measure of goodness of fit. See Sec. 11.5.2.2 for the definition of r^2.

11.5.3.2 Under ideal conditions (i.e., a "perfect" fit of the model to the data), the r^2 will equal 1.00. In order to be an acceptable non-linear calibration, the r^2 must be ≥ 0.99. Alternatively, either of the two methods described in 11.5.4 may be used to determine calibration function acceptability. It is recommended that the resulting calibration curve be inspected by the analyst, as described in Sec. 11.5.4.1.

As noted in Sec. 11.5, whichever of these options is employed, an analyte or surrogate concentration must fall within the calibration range. Analysts are also advised to check both second- and third-order calibration models to ensure that all tangents to the curve within the calibration range are of the same sign and no tangent is zero. Samples with concentrations that exceed the calibration range must be diluted to fall within the range.

11.5.4 Acceptance criteria independent of calibration model

Either of the two procedures described in Secs. 11.5.4.1 and 11.5.4.2 may be used to determine calibration function acceptability for linear and non-linear curves. These include refitting the calibration data back to the model. Both % Error and Relative Standard Error (RSE) evaluate the difference between the measured and the true amounts or concentrations used to create the model.

11.5.4.1 Calculation of the % Error

$$\%\ Error = \frac{x_i - x'_i}{x_i}\ x\ 100$$

where:

x'_i = Measured amount of analyte at calibration level *i*, in mass or concentration units

x_i = True amount of analyte at calibration level *i*, in mass or concentration units.

Percent error between the calculated and expected amounts of an analyte should be ≤ 30% for all standards. For some data uses, ≤50% may be acceptable for the lowest calibration point.

11.5.4.2 Calculation of Relative Standard Error (RSE - expressed as %)

$$RSE = 100 \times \sqrt{\sum_{i=1}^{n}\left[\frac{x_i' - x_i}{x_i}\right]^2 \Big/ (n-p)}$$

where:

x_i = True amount of analyte in calibration level *i*, in mass or concentration units
x'_i = Measured amount of analyte in calibration level *i*, in mass or concentration units
p = Number of terms in the fitting equation
(average = 1, linear = 2, quadratic = 3, cubic = 4)
n = Number of calibration points.

The RSE acceptance limit criterion for the calibration model is the same as the RSD limit for $\overline{CF}$ or $\overline{RF}$ in the determinative method. If the RSD limit is not defined in the determinative method, the limit should be set at ≤20% for good performing compounds and ≤30% for poor performing compounds. A list of known poorly performing compounds can be found in Sec. 16 of this document.

11.5.5 Data transformations

An understanding of the fundamental behavior of the detector may be used to choose a data transformation that will then allow for a simple calibration model. For example, the response of a flame photometric detector in the sulfur mode is known to be proportional to the square of the sulfur concentration. Therefore, using the data system to take the square root of the instrument response or peak height allows for a calibration factor approach rather than a polynomial calibration curve. Instrument response may be transformed prior to any calculations (including integration) subject to the following constraints:

11.5.5.1 Any parameters used in the transformation must be fixed for the calibration and all subsequent analyses and verifications until the next calibration.

11.5.5.2 The transformation model chosen must be consistent with the behavior of the instrument and detector. All data transformations must be clearly defined and documented by the analyst and related back to the fundamental behavior of the detector. In other words, this approach may not be used in the absence of specific knowledge about the behavior of the detector.

11.5.5.3 No transformations should be performed on areas or other results (e.g., the transformation must be applied to the instrument response itself).

11.5.5.4 When the transformed data are used to develop calibration factors, those factors should meet the acceptance criteria described in Sec. 11.5.1, and it is recommended that the resulting calibration "curve" be inspected by the analyst as described in Sec. 11.5.4.

11.5.6 Inspecting the calibration model and recommended corrective actions

Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed acceptance limits for a given calibration. If the criterion is not met by a target analyte, the acceptability of the initial calibration for other analytes that have met their criteria is not invalidated. Information obtained from the initial calibration of targeted analytes not meeting the acceptability criteria may have other uses such as for screening and for estimation of quantitation (see Sec 11.5.6.1), but those uses should still fit the needs of the project objectives.

Whichever calibration model is selected, it is recommended that the model be subjected to an additional check to establish the representativeness of the data that were used to produce the model. This check is the refitting of the calibration data back to the model or the comparison of the calculated amount of each of the standards against the expected amount, as described in Sec. 11.5.4. Criteria for acceptability based upon the additional check would have a similar impact upon the usability of a calibration for quantitation as is discussed in the above paragraph.

11.5.6.1 Corrective action may be needed if the calibration criteria (RSD/r^2 and %Error/RSE) are not met. If <u>any</u> analyte for <u>any</u> calibration standard has a percent error > ±30% as described in Section11.5.4.1, corrective action may be needed. Some recommended courses of action and additional options for modifying the calibration ranges follow. More specific corrective actions that are provided in the applicable determinative methods will supersede those noted in Method 8000. Generally, the calibration should not be used for quantitative analyses of that analyte when the calibration criteria (RSD/r^2 and % Error/RSE) are not met.

11.5.6.2 For all calibration models the following options are allowed. However, if none result in an acceptable calibration, a new initial calibration must be performed.

11.5.6.3 Generally, the first option is to check the instrument operating conditions. The suggested maintenance procedures in Sec. 11.11 may be useful in guiding such adjustments. This option will apply in those instances where a linear instrument response is expected. It may involve some trade-offs to optimize performance across all target analytes. For instance, changes to the operating conditions necessary to achieve linearity for problem compounds may cause the RSD for other compounds to increase, but as long as all analytes meet the RSD limits for linearity, the calibration is acceptable.

If the initial calibration for any analyte does not meet the acceptance criteria (e.g., RSD/RSE > 20% or r^2 < 0.99), the analyst may wish to review the results (proper identification, area counts, calibration or RFs, and RSD/RSE) for those analytes to ensure that the problem is not associated with just one of the initial calibration standards.

11.5.6.4 As a second option, if the problem appears to be associated with a single standard, that one standard may be reanalyzed, to rule out problems due to random error, and the calibration function may be recalculated and reevaluated against the acceptance criteria. Replacing the standard may be necessary in some cases. If the criteria still cannot be met, the entire initial calibration should be performed again.

NOTE: An initial calibration should be considered a single event process and a reanalysis of a calibration standard should be performed immediately to ensure that the reanalysis is still part of the original initial calibration event, and before any samples are analyzed.

11.5.6.5 A third option is to narrow the calibration range by replacing one or more of the calibration standards with standards that cover a narrower range. If linearity can be achieved using a narrower calibration range, document the calibration linearity, and proceed with analyses. Changes to the upper end of the calibration range will affect the need to dilute samples above the range, while changes to the lower end may increase the LLOQ. Consider the regulatory limits or action levels associated with the target analytes when adjusting the lower end of the range. Replacing one or more of the standards is not to be confused with discarding results from a given standard. The minimum number of standards described in Sec. 11.5.3.1 should still be used for calibration.

11.5.6.6 A fourth option is to narrow the calibration range by removing data points from either extreme end of the range and recalculating the calibration function. It is prohibited to remove data points from within a calibration range while still retaining the extreme ends of the calibration range. The minimum number of calibration levels described in Sec. 11.5 should still be met for the model.

NOTE: As noted in Sec. 11.4.1.2, the LLOQ is established by the concentration of the lowest standard analyzed during the initial calibration. Hence, narrowing the calibration range by changing the concentration of the lowest standard will, by definition, change the LLOQ. When the purpose of the analysis is to demonstrate compliance with a specific regulatory limit or action level, the analyst should ensure that the LLOQ is at least one calibration point below the regulatory limit or action level.

If criteria for RSD/RSE/r^2 has been met for the calibration model but the % error of one or more of the individual calibration points at the extreme ends of the calibration range exceeds the criteria described in Sec. 11.5.4.1, the usable range of the calibration may be narrowed to the standards that meet the % error criteria, but the calibration points used to generate the initial curve are retained. The LLOQ becomes the lowest end of the adjusted calibration range. The calibration model should meet the RSD/RSE/r^2 criteria (Secs. 11.5.1 – 11.5.3) and the minimum number of data points (Sec. 11.5.3.1) before this option can be used.

NOTE: This guidance allows the use of a calibration model constructed using all of the data points (with the exception of the highest or lowest point, which may be dropped, but will change linear range) but limits the range for usefulness to only those data points that refit the model within the criteria set in Sec. 11.5.5.1 (i.e., < 30% difference).

11.5.6.7 A fifth alternative is available for target analytes that do not meet the acceptance criteria for the initial calibration. Without reanalysis of standards or manipulations of the model, the initial calibration can be used to estimate quantitation and information from the calibration can be used to verify the identification of target analytes when used to screen samples.

If the initial calibration does not meet the acceptance criteria, it may not be used for quantitative analyses; however, estimates of the quantitation can be made. Estimates of quantitation can be useful when screening for the level of contamination and determining the degree of dilutions that may be necessary when high levels of contamination are encountered. If quantitation estimates for a positively identified analyte are not within the scope of the project DQOs, then an acceptable initial calibration should be prepared for that analyte.

If information from the initial calibration will be used to verify the identification of a targeted analyte for screening purposes, there should be sufficient sensitivity at the screening level to verify identification. Reasonable responses found at the lowest level of the calibration standards may be used as a verification of identity at that level of concentration.

11.6 Retention time windows

Retention time windows are crucial to the identification of target compounds. Absolute retention times are used for compound identification in all GC and HPLC methods that do *not* employ internal standard calibration. Retention time windows are established to compensate for minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. Width of the retention time window should be carefully established to minimize the occurrence of both false positive and false negative results. Tight retention time windows may result in false negatives and/or may cause unnecessary reanalysis of samples when surrogates or spiked compounds are erroneously not identified. Overly wide retention time windows may result in false positive results that may not be confirmed.

The following subsections describe one approach that may be used to establish retention time windows for GC and HPLC methods. Other approaches may be employed, provided the analyst can demonstrate performance appropriate for the intended application.

11.6.1 Before establishing retention time windows, make sure that the chromatographic system is operating reliably and that the system conditions are optimized for the target analytes and surrogates in the sample matrix to be analyzed. Make three injections of all single component standard mixtures and multi-component analytes (such as PCBs) over the course of a 72-hour period. Serial injections or injections over a period of less than 72 hours may result in retention time windows that are too tight.

11.6.2 Record the retention time (in minutes) for each single component analyte and surrogate to three decimal places. Calculate the mean and standard deviation of the three absolute retention times for each single component analyte and surrogate. For multi-component analytes, choose three to five major peaks (see the determinative methods for more details) and calculate the mean and standard deviation of those peaks.

11.6.3 If the standard deviation of the retention times for a target compound is 0.000 (i.e., no difference between the absolute retention times), then the laboratory may either collect data from additional injections of standards or use a default standard deviation of 0.01 minutes. (Recording retention times to three decimal places rather than only two should minimize the instances in which the standard deviation is calculated as 0.000).

11.6.4 Width of the retention time window for each analyte, surrogate, and major constituent in multi-component analytes is defined as ±3 times the standard deviation of

the mean absolute retention time established during the 72-hour period or 0.03 minutes, whichever is greater.

11.6.5 Establish the center of the retention time window for each analyte and surrogate by using the absolute retention time for each analyte and surrogate from the calibration verification standard at the beginning of the analytical shift. For samples run during the same shift as an initial calibration, the retention time of the midpoint standard of the initial calibration should be used.

11.6.6 If the instrument data system is not capable of employing compound-specific retention time windows, then the analyst may choose the widest window and apply it to all compounds. As noted above, other approaches may also be employed, but must be documented by the analyst.

11.6.7 Surrogates are added to each sample, blank, and QC sample and are also contained in each calibration standard. Although the surrogates may be diluted out of certain sample extracts, their retention times in the calibration standards may be useful in tracking retention time shifts. Whenever the observed retention time of a surrogate is outside of the established retention time window, the analyst is advised to determine the cause and correct the problem before continuing analyses.

11.7 Calibration verification

The calibration relationship established during the initial calibration (Sec. 11.5) must be verified at periodic intervals. The process of calibration verification applies to both external standard and internal standard calibration techniques, as well as to linear and non-linear calibration models.

As a general rule, the initial calibration in a SW-846 method must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed using a calibration verification standard prepared at the appropriate level of concern. (Some methods may specify more frequent verifications and recommended standard concentrations). The 12-hour analytical shift begins with the injection of the calibration verification standard (or the MS tuning standard in MS methods). The shift ends after the completion of the analysis of the last sample or standard that can be injected within 12 hours of the beginning of the shift.

If the % Difference (when using average RF calibration) or % Drift (for all other types of calibration) of an analyte is within ±20% of the expected concentration or amount based on the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the calibration curve to quantitate sample results. The ±20% criterion may be superseded in certain determinative methods.

Except where the determinative method contains alternative calibration verification criteria, if the % Drift or % Difference is >± 20%, the initial calibration relationship may no longer be valid.

NOTE: The process of calibration verification is fundamentally different from the approach called "continuing calibration" in some methods from other sources. As described in those methods, the calibration factors or RFs calculated during continuing calibration are used to update the calibration factors or RFs used for sample quantitation. This approach, while employed in other EPA programs, is equivalent to a daily single-point calibration, and is neither appropriate nor permitted in SW-846 chromatographic procedures for trace environmental analyses.

If the calibration does not meet the acceptance criteria, perform any necessary instrument maintenance, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not ± 20%, then a new initial calibration may be necessary.

11.7.1 Calibration verification criteria

Use the equations below to calculate % Drift or % Difference, depending on the procedure described in the determinative method.

$$\%\ \text{Drift} = \frac{\text{Measured Amount}}{\text{True Amount}} \text{x } 100$$

where:

Measured amount = mass or concentration determined by the calibration model

True amount = prepared mass or concentration of the analyte in the standard.

$$\%\ \text{Difference} = \frac{CF_v - \overline{CF}}{\overline{CF}} \times 100\% \quad = \frac{RF_v - \overline{RF}}{\overline{RF}} \times 100$$

where:

CF_v = calibration factor calculated for the calibration verification standard

RF_v = response factor calculated for the calibration verification standard

$\overline{CF}$ = mean calibration factor from the initial calibration

$\overline{RF}$ = mean response factor from the initial calibration

If >10% of the analytes in a multi-analyte method exceed the calibration verification criteria, and instrument maintenance does not correct the problem, then a new initial calibration is necessary. If ≤10% of the analytes exceed the calibration verification criteria, then the initial calibration may still be used, but any detected analytes exceeding the limit must be reported as estimated. Non-detected analytes may be reported if the calibration verification for that specific analyte exceeds the upper acceptance criteria (e.g., >+20%). In order to report non-detected analytes that exceeds the lower acceptance criteria (e.g., <-20%), a sensitivity verification standard at or below the LLOQ should be analyzed in the analytical batch. The analyte should be detected in the LLOQ standard and meet all of the qualitative identification criteria that the laboratory routinely uses (for example, qualifier ions of columns, signal to noise, etc.). In any event, the limitation to no more than 10% of analytes exceeding the calibration verification criteria applies to both detected and non-detected analytes.

11.7.2 Verification of non-linear calibration

Calibration verification of a non-linear calibration is performed using the % Drift calculation and criteria described in Sec. 11.7.1, above.

It may also be appropriate to employ two standards at different concentrations to verify the calibration. One standard should be near the quantitation limit or action limit. Choice of specific standards and concentrations is generally a method- or project-specific consideration.

11.7.3 Calibration verification may be performed using both high and low concentration standards from time to time. This is particularly true when the ECD or ELCD is used. These detectors drift and are not as stable as FID or FPD, and periodic

use of the high and low concentration standards serves as a further check on the initial calibration. Concentrations of these standards should generally reflect those observed in samples.

11.7.4 Additional analyses of the midpoint calibration verification standard during a 12-hour analytical shift are strongly recommended for methods involving external standard calibration. The same evaluation criteria described in Sec. 11.7.1 should be used.

Frequency of verification necessary to ensure accurate measurement is dependent on the detector and the sample matrix. Very sensitive detectors that operate in the sub-nanogram range are generally more susceptible to changes in response caused by column contamination and changes in ambient conditions. Therefore, more frequent verification of calibration (i.e., after every 10 samples) may be necessary for some types of detectors (i.e., electron capture, electrochemical conductivity, photoionization, fluorescence detectors).

Sec. 11.8.2 states that samples analyzed using external standards must be bracketed by periodic analyses of standards that meet the QC acceptance criteria (e.g., calibration and retention time). Therefore, more frequent analyses of standards will minimize the number of sample extracts to be reinjected if the QC limits are violated for the standard analysis. Results from these bracketing standards should meet the calibration verification criteria in Secs. 11.7.1 and 11.7.2 and the retention time criteria in Sec. 11.6. However, if the standard analyzed <u>after</u> a group of samples exhibits a response for an analyte that is <u>above</u> the acceptance limit (i.e., >20%), and the analyte was <u>not</u> detected in any of the previous samples during the analytical shift, then the sample extracts do not need to be reanalyzed, as the verification standard has demonstrated that the analyte would have been detected were it present.

11.7.5 Any method blanks described in the preparative methods (Methods 3500 and 3600) may be run immediately after the calibration verification analyses to confirm that laboratory contamination does not cause false positive results, or at any other time during the analytical shift. If the method blank indicates contamination, then it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples.

11.8 Chromatographic analysis of samples

11.8.1 Introduction of samples or sample extracts into the GC or HPLC varies, depending on the physical and chemical properties of the compound and the solvent matrix. Volatile organics are primarily introduced by purge-and-trap techniques (Method 5030, water and Method 5035, soils). Other techniques include azeotropic distillation (Method 5031), vacuum distillation (Method 5032), headspace (Method 5021), or direct aqueous injection. Use of Method 5021 or another headspace technique may be advisable for screening volatiles in some sample matrices to prevent overloading and contamination of the purge-and-trap system. Semivolatile and non-volatile analytes are introduced by direct or split/splitless injection.

11.8.1.1 Manual injection (GC)

Inject a small volume (i.e., 0.5 - 5 µL) of the sample extract. However, other injection volumes may be used if the analyst can demonstrate appropriate

performance for the intended application. Use of the solvent flush technique is necessary for packed columns.

11.8.1.2 Automated injection (GC)

Automated injectors can provide volumes both larger and smaller than 1 - 2 µL. The analyst should ensure that the appropriate injector design is used for the volume to be injected and that the injection volume is reproducible. Other injection volumes may be used if the analyst can demonstrate appropriate performance for the intended application.

Large Volume Injection (LVI) is the injection of large volumes (greater than 5 µL) into cooled inlets that allow the solvent to be vented while retaining less volatile analytes. LVI is used to increase the sensitivity of the analysis, either to decrease LLOQs or to decrease the amount of sample extracted, or extraction solvent used. This procedure is typically performed with inlets made specifically for this technique. The analyst should ensure that all of the QC requirements of both the preparation and determinative methods are met.

11.8.1.3 Purge-and-trap

Refer to Methods 5000, 5030, or 5035 for details.

11.8.1.4 Manual injection (HPLC)

Inject 10 - 100 µL. This is generally accomplished by overfilling the injection loop of a zero dead-volume injector. Larger volumes may be injected if better sensitivity is needed; however, chromatographic performance may be affected.

11.8.1.5 Automated injection (HPLC)

Inject 10 - 100 µL. Laboratories should demonstrate that the injection volume is reproducible. Larger volumes may be injected if greater sensitivity is needed; however, the solvent of the standards and samples should be matched to the initial mobile phase to avoid chromatographic performance degradation.

11.8.2 All analyses, including field samples, duplicates, MS/MSDs, LCS, method blanks, and any other QC samples are performed during an analysis sequence. The sequence begins with instrument calibration, which is followed by the analysis of sample extracts. Verification of calibration and retention times is necessary no less than once every 12-hour analytical shift. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded. As noted in Secs. 11.5 and 11.7, when employing external standard calibration, run a calibration verification standard at the end of the sequence to bracket the sample analyses. Acceptance criteria for the initial calibration and calibration verification are described in Secs. 11.5 - 11.7.

Analysis of calibration verification standards between every set of 10 samples is strongly recommended, especially for highly sensitive GC and HPLC detectors at sub-nanogram concentrations. Frequent analysis of calibration verification standards helps ensure that chromatographic systems are performing acceptably and that false positives, false negatives and poor quantitation are minimized. Samples analyzed using

external standard calibration should be bracketed by the analyses of calibration standards that meet the QC limits for verification of calibration and retention times. If criteria are exceeded, corrective action should be taken (Sec. 11.11) to restore the system and/or a new calibration curve prepared for that compound and the samples reanalyzed.

Certain methods may also include QC checks on column resolution, analyte degradation, mass calibration, etc., at the beginning of a 12-hour analytical shift.

11.8.3 Sample concentrations are calculated by comparing sample responses with the initial calibration of the system (Sec. 11.5). If sample response exceeds the limits of the initial calibration range, dilute the extract (or sample) and reanalyze. Extracts should be diluted so that all peaks are on scale, as overlapping peaks are not always evident when peaks are off scale. When overlapping peaks cause errors in peak area integration, the use of peak height measurements is suggested.

11.8.4 If chromatographic peaks are masked by the presence of interferences, further sample cleanup or dilution may be necessary. See Method 3600 for guidance.

11.9 Compound Identification

Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation is necessary when the composition of samples is not well characterized. Confirmation techniques include further analysis using a second column with dissimilar stationary phase, GC/MS (full scan or SIM) or HPLC/MS (if concentration permits), GC or HPLC with two different types of detectors, or by other recognized confirmation techniques. For HPLC/UV methods, analyte confirmations at two different UV wavelengths with a UV or UV diode array detector is not recommended because of the broadband nature of UV absorption spectra of many organic compounds. Positive identification of a target analyte using an HPLC/UV method may be confirmed with a different type of detector such as a mass-selective detector or a fluorescence detector at different excitation and emission wavelengths.

When confirmation is made on a second column, that analysis should meet all of the QC criteria described above for calibration, retention times, etc. Confirmation is not needed with GC/MS and HPLC/MS methods.

Confirmation may not be necessary if the composition of the sample matrix is well established by prior analyses as when a pesticide known to be produced or used in a facility is found in a sample from that facility.

Many chromatographic interferences result from coelution of one or more compounds with the analyte of interest or may be the result of the presence of a non-analyte peak in the retention time window of an analyte. Such coelution problems affect quantitation as well as identification and may result in poor agreement between the quantitative results from two dissimilar columns. Therefore, even when the identification has been confirmed on a dissimilar column, the analyst should evaluate the agreement of the quantitative results on both columns, as described in Sec. 11.10.4.

11.10 Calculations

Calculation of sample results depends on the type of calibration (external or internal standard) and the calibration model employed (linear or non-linear). Calculations of the mass of the analyte in the sample aliquot introduced into the instrument can be found in Secs.

11.5.1.3, 11.5.2.3, and 11.5.3. The following sections describe the calculations necessary to obtain the concentrations of analytes in the original sample, based on its volume or weight.

These calculations are provided for illustrative purposes only. Various dilution schemes and conventions for defining final volumes and injection volumes exist and they all cannot be addressed here. The analyst must clearly document and verify all of the calculations that are employed. Specific determinative methods may also contain additional information on how to perform these calculations.

$$Concentration\ in\ \frac{\mu g}{L} = \frac{(X_s)(V_t)(D)}{(V_i)(V_s)}$$

11.10.1 Sample concentration by volume (µg/L), for aqueous samples

where:

X_s = Calculated mass of analyte (in ng) in sample aliquot introduced into instrument. Type of calibration model used determines derivation of x_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3.

V_t = Total volume of concentrated extract (in µL). For purge-and-trap analysis, V_t is the purge volume and will be equal to V_i. Thus, units other than µL may be used for purge-and-trap analyses.

D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. Always dimensionless.

V_i = Volume of extract injected (in µL). The nominal injection volume for samples and calibration must be the same. For aqueous purge-and-trap analysis or direct injection of a liquid sample into a GC or HPLC, V_i will be equal to V_t.

V_s = Volume of aqueous sample extracted or purged (in mL). If units of liters (L) are used for this term, multiply results by 1000 mL/L.

Using the units listed here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

11.10.2 Sample concentration by weight (µg/kg), for solid samples and non-aqueous liquids

$$Concentration\ in\ \frac{\mu g}{kg} = \frac{(X_s)(V_t)(D)}{(V_i)(W_s)}$$

where:

X_s = Calculated mass of analyte (in ng) in sample aliquot introduced into instrument. Type of calibration model used determines derivation of x_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3.

V_t = Total volume of concentrated extract (in µL). For purge-and-trap analysis where an aliquot of solvent (methanol, water, etc.) extract is added to reagent water and purged, V_t is total volume of solvent extract. Also includes any contribution from water present in samples prior to solvent extraction (Sec. 11.10.5).

D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. This value is always dimensionless.

V_i = Volume of extract injected (in µL). The nominal injection volume for samples and calibration standards must be the same. For purge-and-trap analysis where an aliquot of solvent (methanol, water, etc) extract is added to reagent water and purged, V_i is the volume of solvent extract added to reagent water just prior to purging. Dilutions made to the initial volume of solvent extract are accounted for in *D*.

W_s = Weight of sample extracted or purged (in grams). If kg units are used for this term, multiply results by 1000 g/kg.

Using the units listed here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg. See Sec. 11.10.5 for situations in which calculated concentrations may need to be corrected based on the solvent/water dilution effect for extracted volatile organics.

11.10.3 Sample concentration when X_s is expressed as concentration during calibration

As noted in Sec. 11.4, the analyst may develop the calibration using the concentration of analyte and internal standard instead of mass. Using such an approach usually involves expressing concentrations as mass of the analyte or internal standard in the volume injected into the instrument (i.e., ng/µL). Thus, calculations for the final concentration of an analyte in a sample in Secs. 11.10.1 and 11.10.2 must be modified to include the injection volume, V_i, into the term X_s. Therefore, the equation for sample concentration by volume becomes:

$$Concentration\ in\ \frac{\mu g}{L} = \frac{(X_s)(V_t)(D)}{(V_s)}$$

And the equation for sample concentration by weight becomes:

$$Concentration\ in\ \frac{\mu g}{g} = \frac{(X_s)(V_t)(D)}{(W_s)}$$

where V_t, D, V_s, and W_s are the same as in Secs. 11.10.1 and 11.10.2 and

X_s = Calculated concentration of analyte (ng/µL) in the sample. Type of calibration model used determines derivation of X_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3.

Using the units listed here for these terms will result in concentrations in ng/mL, which is equivalent to µg/L, or in ng/g, which is equivalent to µg/kg. See Sec. 11.10.5 for situations in which calculated concentrations may need to be corrected based on the solvent/water dilution effect for extracted volatile organics.

11.10.4 Comparison between results from different columns or detectors

When sample results are confirmed using two dissimilar columns or with two dissimilar detectors, the agreement between the quantitative results should be evaluated after the identification has been confirmed. Large differences in the numerical results from the two analyses may be indicative of positive interferences with the higher of the

results, which could result from poor separation of target analytes, or the presence of a non-target compound. However, they may also result from other causes. Thus, in order to ensure that the results reported are appropriate for the intended application, the analyst should make a formal comparison, as described below.

Calculate the RPD between the two concentrations using the formula below.

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} X\ 100$$

where C_1 and C_2 are concentrations on the two columns and the vertical bars in the numerator indicate the absolute value of the difference. Therefore, RPD is always a positive value.

11.10.4.1 If one result is significantly higher (e.g., >40%), check the chromatograms to see if an obviously overlapping peak is causing an erroneously high result. If no overlapping peaks are noted, examine the baseline parameters established by the instrument data system (or operator) during peak integration. A rising baseline may cause the incorrect integration of the peak for the lower result.

11.10.4.2 If no anomalies are noted, review the chromatographic conditions. If there is no evidence of chromatographic problems, it may be appropriate to report the lower result.

Regardless of the presence or absence of chromatographic problems, the data user must be advised of the disparity between the two results, because the user, not the laboratory, is responsible for ensuring that the most appropriate result is reported or utilized. Under some circumstances, including those involved in monitoring compliance with an action level or regulatory limit, further cleanup of the sample or additional analyses may be needed when the two values in question span the action level or regulatory limit.

11.10.5 Moisture-corrected reporting

Results for solid samples may be reported on the basis of wet weight (as received) or dry weight (moisture-corrected) sample concentration. There are merits to either approach; however, some regulatory limits associated with solid wastes and solid samples are based on the form of the waste as generated, which rarely involves oven-dry solids. As a result, there is no default preference for one form or the other.

The choice of "as received" or moisture-corrected reporting is always a project-specific decision that must be based on knowledge of intended use of the data.

When moisture-corrected reporting is required, concentration results for solid samples calculated in Secs. 11.10.2 and 11.10.3 may be converted to moisture-corrected results as follows:

$$Moisture\ corrected\ concentration = \frac{("As\ received"\ concentration)}{(100 - \%\ moisture)}\ X\ 100$$

where % moisture is determined as described in the specific sample preparation or determinative method, typically by drying an aliquot of the sample at 105 °C overnight. Percent moisture is calculated as follows:

$$\% \, Moisture = \frac{(g \; of \; \text{"as received"} \; sample) - (g \; of \; dry \; sample)}{g \; of \; sample} \; X \; 100$$

The % moisture determination may also be called % solids in some methods. In this case, percent solids should be subtracted from 100, in order to attain % moisture as noted in the above moisture-corrected calculation. Units for the final results will be the same, regardless of the % moisture calculation.

Except when the sample is completely dry (i.e., the % moisture equals 0), moisture-corrected results will always be higher than "as received" results. In the absence of project-specific requirements, it may be most appropriate to report results on the "as received" basis of the sample and provide the % moisture for each sample. This will allow the data user to convert the results from one form to another, as needed. The approach used must be clearly described for the data user.

In volatile organic analysis, solid samples with significant moisture content (>10%) that are extracted prior to analysis in a water-miscible solvent such as methanol, are diluted by the total volume of the solvent/water mixture. The total mixture volume can only be calculated based on the sample moisture present as determined by the % moisture determination. This total volume is then expressed as V_t in the sample concentration calculations provided in Secs. 11.10.2 and 11.10.3. Therefore, in order to report results for volatile analysis of samples containing significant moisture content on either "as received" or “moisture-corrected” basis, the calculated concentration must be corrected using the total solvent/water mixture volume represented as V_t. This total solvent/water volume is calculated as follows:

$$\frac{\mu L \; solvent}{water V_t} = \left((mL \; of \; solvent) + \frac{(\% \; Moisture \; \text{x} \; g \; of \; sample)}{100} \right) \text{x} \; 1000 \; \mu L/mL$$

When the sample moisture content is >10%, it is recommended that the calculated concentrations of volatile samples that are extracted in a water-miscible solvent such as methanol be corrected for the solvent/water dilution effect. Potential underreporting of volatile concentrations is more pronounced as % moisture increases.

11.11 Suggested chromatographic system maintenance

Following is a list of corrective measures that may be employed to prevent or ameliorate the deterioration of chromatographic performance. This list is by no means comprehensive, and analysts should develop expertise in troubleshooting their specific instruments and analytical procedures. Manufacturers of chromatographic instruments, detectors, columns, and accessories generally provide detailed information regarding the proper operation and limiting factors associated with their products. Reading and reviewing this information cannot be overemphasized.

11.11.1 GC preventive maintenance and corrective action

To prevent or ameliorate deterioration of chromatographic performance, analysts should perform routine maintenance activities on the GC inlet, column, and gas delivery system.

11.11.1.1 Inlet maintenance

Appropriate injector liners should be installed and replaced as necessary to maintain chromatographic performance. Injection port septa should also be changed frequently enough to prevent retention time shifts of target analytes and peak tailing. Over-tightening the septum nut can cause the inlet to leak. The schedule for changing inlet liners and septa is dependent on the operation of the injection system, the nature of samples and parameters tested, and acceptance criteria in the reference method.

If chromatographic performance or ghost peaks are still a problem after performing these inlet maintenance steps, replacing the seal in the bottom of the inlet or cleaning and deactivating the metallic surfaces of the injection port itself may be necessary. Deactivation of the injection port necessitates the use of toxic reagents and should only be performed by knowledgeable personnel according to the instrument manufacturer's instructions.

11.11.1.2 Column maintenance

Capillary columns are reliable and easy to use but overheating and exposure to oxygen can cause damage. Install and condition the column as recommended by the manufacturer and flush the column with carrier gas before conditioning. Avoid contact between the capillary column and the metal surfaces in the GC oven or heating above the maximum column temperature.

Poor chromatographic performance may also be observed when the head of the GC column is contaminated with high-boiling material. Removing as much as 0.5 - 1 m from the injector side of the capillary column may restore chromatographic performance. If clipping the head of the column does not restore performance, replacement of the column may be necessary. Using a guard column may extend column life.

11.11.1.3 Gas delivery system maintenance

Analysts should periodically ensure that proper flow control is maintained. A search for leaks using an electronic leak detector or by isolating and pressure testing various parts of the delivery system may be conducted; static pressure tests may also be performed, or other appropriate measures taken. Electronic pressure controller flow rates should also be checked regularly (with both the injector and the oven heated) using a bubble meter or other appropriate device. A leak in the gas delivery system and/or change in delivery pressure can lead to retention time shifts of the target analytes in the GC chromatograms. If this is observed, corrective action should be taken. Monitoring retention times in standards over time can help to ensure that

11.11.2 HPLC preventive maintenance and corrective action

Band broadening occurs whenever there is a dead volume between the injector and detector. Therefore, plumbing connections should be of minimum length and diameter, and ferrules should be properly positioned on the tubing to minimize dead volume.

11.11.2.1 Injection port maintenance

Filtration of extracts and injection of solvent mixtures miscible with the mobile phase can help minimize solvent-related problems. Otherwise, contamination of subsequent injections may occur when the extract contains material that is not soluble in the mobile phase. Injectors also need maintenance, as the surfaces that turn past each other tend to wear down over time, potentially causing leaks. Injection loops are easily changed, but analysts must ensure that the compression fittings are properly installed to prevent leaks.

11.11.2.2 Column maintenance

Use of high quality columns that are uniformly packed with the appropriate particle size and bonded phase will result in optimal chromatographic performance. Column temperatures may be regulated by the use of temperature control ovens to ensure reproducibility of retention times.

Lifetime and performance of HPLC columns can be improved through proper maintenance. Sample extracts should be filtered prior to analysis, and care should be taken to ensure that storage conditions (e.g., freezing) do not cause subsequent precipitation of solids in the extracts prior to analysis. Guard columns should be used when dirty samples are analyzed because HPLC columns can become contaminated with particulates or insoluble materials. If degradation of resolution or changes in back pressure are observed, the replacing the guard column if one is installed may restore performance. Columns should not be stored dry or containing strong buffers, and they should be replaced when performance degrades (e.g., significant band broadening, peak splitting, or loss of chromatographic resolution occurs).

11.11.2.3 Mobile phase and pump maintenance

Pumping systems should deliver reproducible gradients at a uniform flow rate. Pumping flow rates can be checked by collecting solvent into a graduated cylinder for a designated time period.

Air bubbles tend to cause an erratic baseline and, in the case of low-pressure mixing, bubbles can cause the pump to cavitate. Therefore, HPLC solvents should be degassed prior to use.

Non-reproducible gradients can result from deterioration in pump performance and can cause unacceptable variation in retention times from run to run. Mobile phase solvents should be filtered to remove particles that cause pump piston wear. Seals in the HPLC pumps should be replaced regularly. Use of strong buffers or solvents such as tetrahydrofuran can significantly shorten the lifetime of pump seals and should be avoided where practical.

Small changes in the composition or pH of the mobile phase can have a

significant effect on retention times. Buffering the mobile phase may help make the pH more reproducible from preparation to preparation, as long as it doesn't conflict with the method or cause other problems with the analysis. Precise measurement of reagents and care in mixing the mobile phase may help ensure consistency from one preparation to the next. A solvent mixer may be the best way to ensure reproducibility of the mobile phase over time.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 See Sec. 11.0 and the appropriate determinative method for information regarding data analysis and calculations.

12.2 Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. Data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on a project-specific basis and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 Refer to individual determinative methods for performance data examples and guidance.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult: http://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Laboratories are urged to protect air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations and complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land

disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available at: http://www.labsafety.org/FreeDocs/WasteMgmt.pdf

16.0 REFERENCES

16.1 For further information regarding these methods, review Methods 3500, 3600, 5000, the individual sample preparative, cleanup and determinative methods, and Chapter One.

16.2 Three references for poorly performing compounds in general are shown below.

Department of Defense:
"*Quality Systems Manual for Environmental Laboratories*", Version 5.1, Jan 2017
https://www.denix.osd.mil/edqw/documents/documents/qsm-version-5-1-final/

EPA:
A list of typical quantitation limits for commonly analyzed volatile and semivolatile compounds can be found in Appendix C of the EPA Contract Laboratory Program Statement of Work for Organic Superfund Methods Multi-Media, Multi-Concentration SOM02.4, Oct 2016: https://www.epa.gov/clp/epa-contract-laboratory-program-statement-work-organic-superfund-methods-multi-media-multi-1

Department of Defense:
"Environmental Data Quality Workgroup (EDQW) Laboratory Control Sample Control Limits Study", July 2013 at: https://www.denix.osd.mil/edqw/home/edqw-home-documents/documents/final-lcs-study-july-2013/

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

There are no tables or figures associated with this method.

APPENDIX A
(Summary of Revisions to Method 8000D from Revision 4, July 2014)

1. The revision number was changed to 5 and the footer date updated to March 2018. A table of contents was added.

2. Sec. 11.5 was updated to state that calibration options listed are also appropriate for GC/MS and LC/MS instruments.

3. The references contained in Sec. 16, which listed poor performing compounds in volatile and semivolatile analyses, were replaced with a link to the CLP Appendix C, which lists common reporting levels used for many compounds for volatiles and semivolatiles. The links for references to DOD and EPA sources were updated to the current versions.

4. Tables and graphics in this method were updated to be 508 compliant.

5. The ACS document in Sec. 14 was updated.

6. The reference in Sec. 15 was updated.