METHOD 8000B

DETERMINATIVE CHROMATOGRAPHIC SEPARATIONS

1.0 SCOPE AND APPLICATION

1.1 Method 8000 is not a determinative method but instead provides guidance on analytical chromatography and describes calibration and quality control requirements that are common to all SW-846 chromatographic methods. Apply Method 8000 in conjunction with all SW-846 determinative chromatographic methods. The methods include, but are not limited to, the following:

Method Number	Analytes	Chromatographic Technique (see Sec. 1.5)	Detector
7580	White phosphorus (P_4)	GC, capillary column	NPD
8011	EDB, DBCP	GC, capillary column	ECD
8015	Nonhalogenated 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 ketones	GC, capillary column	ECD
8100	PAHs	GC, packed & capillary column	FID
8111	Haloethers	GC, capillary column	ECD
8121	Chlorinated hydrocarbons	GC, capillary column	ECD

Method Number	Analytes	Chromatographic Technique (see Sec. 1.5)	Detector
8131	Aniline and selected derivatives	GC, capillary column	NPD
8141	Organophosphorus pesticides	GC, capillary column	FPD, NPD, ELCD
8151	Acid herbicides	Derivatize; GC, capillary column	ECD
8260	Volatiles	GC, capillary column	MS
8270	Semivolatiles	GC, capillary column	MS
8275	Semivolatiles	Thermal extraction/GC	MS
8280	Dioxins and Dibenzofurans	GC, capillary column	Low resolution MS
8290	Dioxins and Dibenzofurans	GC, capillary column	High resolution MS
8310	PAHs	HPLC, reverse phase	UV, Fluorescence
8315	Carbonyl compounds	Derivatize; HPLC	Fluorescence
8316	Acrylamide, acrylonitrile, acrolein	HPLC, reverse phase	UV
8318	N-Methyl carbamates	Derivatize; HPLC	Fluorescence
8321	Extractable nonvolatiles	HPLC, reverse phase	TS/MS, UV
8325	Extractable nonvolatiles	HPLC, reverse phase	PB/MS, UV
8330	Nitroaromatics and nitramines	HPLC, reverse phase	UV
8331	Tetrazene	HPLC, ion pair, reverse phase	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
ECD = Electron capture detector
EDB = Ethylene dibromide
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
NPD = Nitrogen/phosphorous detector
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: gas chromatography (GC) and high performance liquid chromatography (HPLC).

1.2.1 Gas chromatography (more properly called gas-liquid chromatography) is the separation technique of choice for organic compounds which can be volatilized without being decomposed or chemically rearranged.

1.2.2 High performance liquid chromatography (HPLC) is a separation technique useful for semivolatile and nonvolatile 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. Because the solvents are delivered under pressure, the technique was originally designated as high pressure liquid chromatography, but now is commonly referred to as high performance liquid chromatography.

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., long retention time), while compounds that remain in the mobile phase elute quickly (i.e., short retention time).

1.3.1 The mobile phase for GC is an inert gas, usually helium, and the stationary phase is generally a silicone oil or similar material.

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.

1.4 A number of specific GC and LC techniques are used for environmental and waste analyses. The specific techniques are distinguished by the chromatographic hardware or by the 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.

1.4.1.1 Packed columns are typically made from glass or stainless steel tubing and generally are 1.5 - 3 m long with a 2 - 4 mm ID, and filled with small particles (60-100 mesh diatomaceous earth or carbon) coated with a liquid phase.

1.4.1.2 Capillary columns are typically made from open tubular glass capillary columns that are 15 - 100 m long with a 0.2 - 0.75 mm ID, and coated with a liquid phase. Most capillary columns are now made of fused silica, although glass columns are still sold for the analysis of volatiles. Capillary columns are inherently more efficient than packed columns and have replaced packed columns for most SW-846 applications.

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

1.4.2.1 Partition chromatography is the basis of reverse phase HPLC separations. Analytes are separated on a hydrophobic column using a polar mobile phase pumped at high pressure (800 - 4000 psi) through a stainless steel column 10 -

25 cm long with a 2 - 4 mm ID and packed with 3 - 10 µm silica or divinyl benzene-styrene particles.

1.4.2.2 Ion exchange chromatography is used to separate ionic species.

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 required, then 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 the sensitivity, accuracy, precision, frequency of false positives, and frequency of false negatives during analysis. The laboratory must demonstrate that an alternate chromatographic procedure provides performance that satisfies the analytical requirements of the specific application for which it is being used. Such demonstrations should be performed using the procedures outlined in Secs. 8.2 to 8.5 of this method and those in Chapter One.

1.5.2 In addition, laboratories must be cautious whenever the use of two dissimilar columns is included in a method for confirmation of identification. 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 gas chromatographic conditions are changed, retention times and analytical separations are often affected. For example, increasing the GC oven temperature changes the 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 film thickness or percent loading for packed columns), or alternate 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 changed by adjusting the composition of the solvent mixture being pumped through the column. In reverse phase HPLC, increasing the ratio of methanol (or acetonitrile) to water shortens retention times. HPLC retention times can also be changed by selecting a column with (1) a different length, (2) an alternate bonded phase, or (3) a different particle size (e.g., smaller particles generally increase column resolution). 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. HPLC methods are particularly sensitive to small changes in chromatographic conditions, including temperature. HPLC column temperature control ovens should be used to maintain constant retention times since ambient laboratory temperatures often fluctuate throughout the course of a day.

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 being 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 co-elution of target analytes, false negatives due to retention time shifts, and false positives and quantitation errors due to co-eluting 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 of the chromatographic methods includes a list of the compounds that it may be used to determine. The lists in some methods are lengthy and it may not be practical or appropriate to attempt 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) which may be applicable to those analytes.

1.10 Prior to employing this method, analysts are advised to consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the data quality objectives or needs for the intended use of the data.

2.0 SUMMARY OF METHOD

Method 8000 describes general considerations in achieving chromatographic separations and performing calibrations. Method 8000 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. 3.1 of this method provides general guidance on minimizing contamination, including cross-contamination between samples. Sample screening procedures are strongly recommended, and discussed in Sec. 3.2.

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 (see Secs. 3.3 and 8.3).

2.3 Secs. 3.4 and 3.5 provide information on the effects of chromatographic interferences.

2.4 Sec 4.0 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. 7.5 discusses specific procedures and calculations for both linear and non-linear calibration relationships. The continued use of any chromatographic procedure requires a verification of the calibration relationship, and procedures for such verifications are described in this method as well (see Sec. 7.7).

2.6 The identification of target compounds by any chromatographic procedure is based, at least in part, on retention times. Sec. 7.6 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 The calculations necessary to derive sample-specific concentration results from the instrument responses are common to most of the analytical methods listed in Sec. 1.1. Therefore, Sec. 7.10 of Method 8000 contains a summary of the commonly used calculations.

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. 7.11.

2.9 Most of the methods listed in Sec. 1.1 employ a common approach to quality control (QC). While some of the overall procedures are described in Chapter One, Sec. 8.0 describes routinely used procedures for calibration verification, instrument performance checks, demonstrating acceptable performance, etc.

2.10 Before performing analyses of specific samples, analysts should determine acceptable recovery ranges for all target analytes of interest in the type of matrices to be tested. These procedures are described in Secs. 8.4, 8.5, and 8.7. 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 detection limits for the analytes of interest using the procedures in Chapter One.

3.0 INTERFERENCES/CHROMATOGRAPHIC PERFORMANCE

3.1 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed in sequence. To reduce the potential for carryover, the sample syringe or purging device must be thoroughly rinsed between samples with an appropriate solvent. Purge and trap devices or headspace devices should be thoroughly baked out between samples. Where practical, samples with unusually high concentrations of analytes should be followed by a solvent blank or by an analysis of organic-free reagent water to check for cross-contamination. If the target compounds present in an unusually concentrated sample are also found to be present in the subsequent samples, the analyst must demonstrate that the compounds are not due to carryover. Conversely, if those target compounds are <u>not</u> present in the subsequent sample, then the analysis of a solvent blank or organic-free reagent water is not necessary.

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 may also be employed, provided that the laboratory can demonstrate that they are effective in removing contaminants.

3.2 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. This is particularly true for GC/MS. Eliminating this contamination can require significant time and effort in cleaning the instruments, time that cannot be spent analyzing samples. The most reliable procedure for ensuring minimum down time during the GC/MS analysis of samples is to screen samples by some other 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). Samples to be analyzed for semivolatiles can be screened using GC/FID. Other screening methods are also acceptable. The analyst should use the 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.

3.3 One of the most important measures of chromatographic performance is resolution, the separation of chromatographic peaks (peak separation/average peak width). Peak separations are facilitated by good column efficiency (i.e., narrow peak widths) and good column selectivity (i.e., analytes partition differently between the mobile and stationary phases).

3.3.1 The goal of analytical chromatography is to separate sample constituents within a reasonable time. Baseline resolution of each target analyte from co-extracted materials provides the best quantitative results, but is not always possible to achieve.

3.3.2 In general, capillary columns contain a greater number of theoretical plates than packed columns. (A theoretical plate is a surface at which an interaction between the sample components and the stationary phase may occur). As a result, capillary columns generally provide more complete separation of the analytes of interest. 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.

3.3.3 The ability to resolve individual compounds is generally the limiting factor for the number of analytes that can be measured using a single procedure. Some procedures, particularly Method 8081 (Organochlorine Pesticides), Method 8082 (PCBs), and Method 8141 (Organophosphorus Pesticides), list analytes that may not all be resolved from one another. Therefore, while each of these methods is suitable for the listed compounds, they may not be suitable to measure the entire list in a single analysis. In addition, some methods include analytes that are isomers or closely related compounds which are well-known as co-eluting or are not completely separable. In these instances, the results should be reported as the sum of the two (or more) analytes. Laboratories should demonstrate that target analytes are resolved during calibration and satisfy the requirements in Sec. 8.3, or should report the results as "totals" or "sums" (e.g., m+p-xylene). Methods that utilize mass spectrometry for detection are less affected by resolution problems, because overlapping peaks may often be mass-resolved. However, even mass spectrometry will not be able to mass resolve positional isomers such as m-xylene and p-xylene if the compounds co-elute.

3.4 Elevated chromatographic baselines should be minimized or eliminated during these analyses. Baseline humps can usually be reduced or eliminated by the application of appropriate sample clean-up (see Method 3600), extract dilution, the use of pre-columns and/or inserts, or use of a selective detector. Integration of "hump-o-grams" can result in significant quantitative errors. When elevated baselines are observed during the analysis of blanks and standards, the chromatographic system should be considered contaminated. This contamination may be the result of impure carrier gas, inadequate gas conditioning, septum bleed, column oxidation, and/or pyrolysis products in the injector or column. Such contamination is unacceptable and should be addressed through a program of preventive maintenance and corrective action.

3.5 GC preventive maintenance and corrective action

Poor GC performance may be expected whenever a chromatographic system is contaminated with high-boiling materials, particularly in the injector. Analysts should perform routine maintenance, including replacement of septa, cleaning and deactivating injector liners, and removing as much as 0.5 - 1 m from the injector side of a capillary column.

If chromatographic performance or ghost peaks are still a problem, cleaning of the metallic surfaces of the injection port itself may be necessary. Capillary columns are reliable and easy to use, but several specific actions are necessary to ensure good performance.

3.5.1 Contact between the capillary column and the wall of the GC oven can affect both chromatographic performance and column life. Care should be taken to prevent the column from touching the oven walls.

3.5.2 Care should be taken to keep oxygen out of capillary columns.

3.5.3 Septa should only be changed after the oven has cooled.

3.5.4 Columns should be flushed with carrier gas for 10 minutes before reheating the oven.

3.5.5 Carrier gas should be scrubbed to remove traces of oxygen and scrubbers should be changed regularly.

3.5.6 Carrier gas should always be passed through the column whenever the oven is heated.

3.6 HPLC preventive maintenance and corrective action

HPLC band broadening results from improper instrument setup or maintenance. Band broadening results whenever there is a dead volume between the injector and the detector. Therefore, plumbing connections should be of minimum length and diameter, and ferrules should be properly positioned on the tubing to minimize dead volume.

3.6.1 Columns should not be subjected to sudden physical stress (e.g., dropping) or solvent shocks (e.g., changing solvents without a gradient).

3.6.2 Columns can become contaminated with particulates or insoluble materials. Guard columns should be used when dirty samples are analyzed.

3.6.3 High quality columns are packed uniformly with small uniform diameter particles with a minimum number of free silol groups. Use of such columns will result in optimum chromatographic performance.

3.6.4 Columns should be replaced when performance degrades (e.g., significant band broadening, peak splitting, or loss of chromatographic resolution occurs).

3.6.5 Pumping systems should deliver reproducible gradients at a uniform flow rate. Rates can be checked by collecting solvent into a graduated cylinder.

3.6.6 Column temperatures should be regulated by the use of column temperature control ovens to ensure reproducibility of retention times.

3.6.7 Small changes in the composition or pH of the mobile phase can have a significant effect on retention times.

4.0 APPARATUS AND MATERIALS

4.1 GC inlet systems

4.1.1 Volatile organics

Volatile organic analytes are introduced into a GC through a purge-and-trap system, by direct injection, or by other devices. 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.

4.1.2 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 set up and maintenance of the injector port is necessary to achieve acceptable performance with GC methods. Septa should be changed frequently enough to prevent retention time shifts of target analytes and peak tailing. The schedule for such septa changes is dependent on the quality of the septa, the sharpness of the needle, and the operation of the injection system. Appropriate injector liners should be installed, and liners should be cleaned and deactivated (with dichlorodimethylsilane) regularly.

4.1.3 Injector difficulties include the destruction of labile analytes and discrimination against high boiling compounds in capillary injectors.

4.1.3.1 Packed columns and wide-bore capillary columns (> 0.50 mm ID) should be mounted in 1/4-inch injectors. An injector liner is needed for capillary columns.

4.1.3.2 Narrow-bore capillary columns ($\leq$ 0.32 mm ID) should be mounted in split/splitless (Grob-type) injectors. Split/splitless injectors require automated valve closures that direct most of the flow (and sample) onto the head of the analytical column. After 30 - 45 seconds, the split valve is opened, so that most of the flow is vented during analysis, thus eliminating the solvent tail, and 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.

4.1.3.3 Cool on-column injection allows the analysis of labile compounds that degrade on packed columns and in split/splitless injectors.

4.2 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 SW-846 analyses must deliver a precisely metered gas flow at a rate appropriate for the GC column mounted in the instrument.

4.2.1 Most GCs have restrictors built into flow controllers. These restrictors are used to provide precise flow at the carrier gas flow rate specified in the method (e.g., use <20 mL/min restrictors for wide-bore capillary methods). Carrier gas flow rates should be checked regularly (with both the injector and the oven heated) using a bubble meter or other appropriate procedure.

4.2.2 Cylinder pressures should also be regulated properly. Manifold pressures must be sufficiently large 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. Analysts should spend time each week conducting preventative maintenance in order to ensure that proper flow control is maintained. One needs to search for leaks using a helium tester or soap solution at each connector in the gas delivery systems. Analysts should routinely conduct preventive maintenance activities, including those designed to ensure proper flow control and to identify potential leaks in the gas delivery system. The search for leaks may be conducted with a helium leak tester, soap solutions, performing static pressure tests, or other appropriate measures.

4.2.3 Carrier gas should be of high purity and should be conditioned between the cylinder and the GC to remove traces of water and oxygen. Scrubbers should be changed according to manufacturers recommendations. Gas regulators should contain stainless steel diaphragms. Neoprene diaphragms are a potential source of gas contamination, and should not be used.

4.3 Gas chromatographic columns

Each determinative method in SW-846 provides a description of a chromatographic column or columns with associated performance data. Other packed or capillary (open-tubular) columns may be substituted in SW-846 methods to improve performance if (1) the requirements of Secs. 8.3 and 8.4 are satisfied, and (2) target analytes are sufficiently resolved from one another and from co-extracted interferences to provide data of the appropriate quality for the intended application.

4.3.1 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).

4.3.2 Longer columns can resolve more analytes, as resolution increases as a function of the square root of column length.

4.3.3 Increasing column film thickness or column loading increases column capacity and retention times.

4.3.4 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.

4.3.5 Columns used for SW-846 analyses should be installed properly. Column ends should be cut square. Contaminated ends should be trimmed off, and columns should be placed through ferrules before they are trimmed. Columns should not touch the walls of the GC oven during analysis, and the manufacturer's column temperature limits should not be exceeded.

4.3.6 Septa should be changed regularly and septum nuts should not be overtightened. Oxygen should not be introduced into a hot column and carrier gas should be passed through a column whenever it is heated. New columns, particularly packed columns, should be conditioned prior to analyzing samples.

4.4 GC detectors

Detectors are the transducers that respond to components that elute from a GC column and produce the electrical signal that is used for quantitative determinations. SW-846 analyses are conducted using selective detectors or mass spectrometers 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. The transfer lines between the GC and an MS detector should be maintained at a temperature above the highest column temperature, or as specified by the instrument manufacturer, to prevent condensation.

4.5 HPLC injectors

Liquids are essentially non-compressible, 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 (generally 10-100 µL) 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 turning the valve 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.

4.5.1 When the extract is highly viscous, a pressure spike results which can automatically shut off the HPLC pump.

4.5.2 Contamination of subsequent injections may occur when the extract contains material that is not soluble in the mobile phase.

4.5.3 Injection loops are easily changed but analysts must ensure that the compression fittings are properly installed to prevent leaks. Injectors require maintenance, as the surfaces that turn past each other do wear down.

4.6 HPLC pumps

The mobile phase used for HPLC must be accurately pressurized before it enters the injector. HPLC pumps are generally capable of delivering solvent at 5000 psi with excellent precision. The rate of delivery depends on the column that is used for the separation. Most environmental methods recommend flow rates of 0.25-1.0 mL/min. Flow rates should be checked by collecting column effluent in a graduated cylinder.

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, the formation of bubbles in the mobile phase, or non-reproducible gradients.

4.6.1 Air bubbles result in 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.

4.6.2 Non-reproducible gradients can result in significant changes in retention times from run to run.

4.6.3 HPLC solvents should be filtered to remove particles that cause pump piston wear. HPLC pump maintenance includes replacing seals regularly. (Use of strong buffers or solvents like tetrahydrofuran can significantly shorten the lifetime of pump seals.) Pumps should deliver solvent with minimal pulsation.

4.7 HPLC Columns

These columns must be constructed with minimum dead volume and a narrow particle size distribution. HPLC columns are generally constructed of stainless steel tubing and are sealed with compression fittings. Manufacturers provide columns that are bonded with different alkyl groups (e.g., C_{18}, cyano, TMS), have different percent carbon loading, are packed with different particle sizes (3-10 µm), and are packed with particles of different pore size (smaller pores mean greater surface area), or are of different dimensions.

4.7.1 Columns with higher percent loading have the capacity to analyze somewhat larger samples, but extremely high loadings may contribute to problems with the particle beam MS interface.

4.7.2 Columns with free silol groups show less tailing of polar materials (e.g., amines).

4.7.3 A smaller particle (and pore) size generally gives better resolution, higher back pressure, and smaller sample capacity. Columns with 3 µm particle size may have short lifetimes when they are used for the analysis of complex waste extracts.

4.7.4 Improvements in column packing have resulted in 10 and 15 cm columns that provide the separating power necessary for most environmental and waste analyses.

4.7.5 Internal diameters of columns used for environmental and waste analysis are generally 2-5 mm. Narrower columns are called microbore columns. While they provide better separations, they become fouled more easily.

4.7.6 The lifetime and performance of HPLC columns can be improved through proper maintenance. Analysts should filter sample extracts, use compatible guard columns, check for clogged frits and for column voids. Columns should not be stored dry or containing strong buffers.

4.8 HPLC column temperature control ovens

HPLC retention times are much 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 maintain consistent retention times throughout the course of an HPLC analysis. Normal oven operating temperature would be 3-5°C above ambient laboratory temperature.

4.9 HPLC detectors

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

4.10 Data systems

Raw chromatographic data have to be reduced in order to provide the quantitative information required by analysts. The use of sophisticated data systems is strongly recommended for SW-846 chromatographic methods. The ability to store and replot chromatographic data is invaluable during data reduction and review. Organizations should establish their priorities and select the system that is most suitable for their applications.

4.11 Supplies

Chromatographers require a variety of supplies. The 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, and syringes, and replacement parts for instruments.

4.11.1 Laboratories performing GC analyses also require 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.

4.11.2 Laboratories performing HPLC analyses require high purity solvents, column packing material, frits, 1/16-inch tubing, appropriate ferrules, solvent filtration apparatus, and solvent degassing apparatus.

5.0 REAGENTS

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

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

Refer to Chapter Four, Organic Analytes, Sec. 4.1, for information on sample collection, preservation and handling procedures. Additional information may be found in some of the individual sample extraction, preparation, and determinative methods.

7.0 PROCEDURE

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

7.1 Extraction

The 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.

7.2 Cleanup and separation

The 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 (such as ground water samples) may not require extensive cleanups, the analyst should carefully balance the time savings gained by skipping cleanups against the potential increases in instrument down time and loss of data quality that can occur as a result.

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

Chromatographic performance is demonstrated by the resolution of standards and the ability to model the response of the detector during calibration, and by the sensitivity, accuracy, precision, frequency of false positives, and frequency of false negatives during analysis. If the laboratory employs an alternative chromatographic procedure or alternative conditions, then the laboratory must demonstrate that the performance satisfies the analytical requirements of the specific application for which the alternative chromatographic procedure is being used. Such demonstrations should be performed using the procedures outlined in Secs. 8.2 to 8.5 of this method and those in Chapter One.

7.4 Initial Calibration

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

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, among them, simplicity and ease of use. Unfortunately, given the advent of new detection techniques and the fact that many techniques cannot be optimized for all of 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.

The initial calibration for SW-846 chromatographic methods involves the analysis of standards containing the target compounds at a minimum of five different concentrations covering the working range of the instrument. In order to produce acceptable sample results, the response of the instrument must be within the working range established by the initial calibration. The 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. Analysts are advised that it may be necessary to prepare calibration standards that cover concentration ranges that are appropriate for specific projects or type of analyses. For instance, the analyst should not necessarily expect to be able to perform a calibration appropriate for sub-ppb level analyses and also use the same calibration data for high-ppb or ppm level samples.

The specific options for evaluating the initial calibration are described in Sec. 7.5. The remainder of this section describes the preparation of calibration standards, the use of external and internal standard calibrations, and the calculation of both calibration factors and response factors.

7.4.1 Calibration standards are prepared using the procedures indicated in Sec. 5.0 of the determinative method of interest. However, the general procedure is described here.

7.4.1.1 For each analyte and surrogate of interest, prepare calibration standards at a minimum of five different concentrations by adding volumes of one or more stock standards to volumetric flasks and diluting to volume with an appropriate solvent.

7.4.1.2 The lowest concentration calibration standard that is analyzed during an initial calibration establishes the method quantitation limit based on the final volume of extract (or sample) described in the preparative method or employed by the laboratory.

7.4.1.3 The other concentrations should define the working range of the detector or correspond to the expected range of concentrations found in actual samples that are also within the working range of the detector.

7.4.1.4 For each analyte, at least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project, which may include establishing compliance with a regulatory or action limit.

7.4.1.5 Given the 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 five solutions at different concentrations. The initial calibration will then involve the analysis of each of these sets of five standards.

7.4.1.6 Once the standards have been prepared, the initial calibration begins by establishing chromatographic operating parameters that provide instrument performance equivalent to that documented in Sec. 7.0 of the determinative method of interest, or that is appropriate for the data quality objectives of the intended application.

7.4.2 External standard and internal standard calibration techniques

The chromatographic system may be calibrated using either the external standard or the internal standard techniques described below. General calibration criteria are provided in this section for GC and HPLC procedures using non-MS detection. The applicable calibration procedures for GC/MS (e.g., Methods 8260, 8270, 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 special guidance on calibration that is specific to that method.

Regardless of whether external standard or internal standard calibration is used, introduce each calibration standard into the instrument using the same technique that is used to introduce the actual samples into the gas chromatograph (e.g., 1-3 µL injections for GC methods, 10-100 µL injections for HPLC methods, purge-and-trap techniques for volatiles, etc.). Tabulate peak area or height responses against the mass or concentration injected, as described below.

7.4.2.1 External standard calibration procedure

External standard calibration involves comparison of instrument responses from the sample to the responses from the target compounds in the calibration standards. Sample peak areas (or peak heights) are compared to peak areas (or heights) of the standards. The ratio of the detector response to the amount (mass) of analyte in the calibration standard is defined as the calibration factor (CF).

$$CF = \frac{\text{Peak Area (or Height) of the Compound in the Standard}}{\text{Mass of the Compound Injected (in nanograms)}}$$

For multi-component analytes, see the appropriate determinative method for information on which areas to employ.

The CF can also be calculated using the concentration of the standard rather than the mass in the denominator of the equation above. However, the use of concentrations in CFs will require changes to the equations that are used to calculate sample concentrations (see Sec. 7.10.1.1).

7.4.2.2 Internal standard calibration procedure

Internal standard calibration involves the comparison of instrument responses from the target compounds in the sample to the responses of specific standards added to the sample or sample extract prior to injection. The ratio of the peak area (or height) of the target compound in the sample or sample extract to the peak area (or height) of the internal standard in the sample or sample extract is compared to a similar ratio derived for each calibration standard. The ratio is termed the response factor (RF), and may also be known as a relative response factor in other methods.

In many cases, internal standards are recommended in SW-846 methods. These recommended internal standards are often brominated, fluorinated, or stable isotopically labeled analogs of specific target compounds, or are closely related compounds whose presence in environmental samples is highly unlikely. If internal standards are not recommended in the method, then the analyst needs to select one or more internal standards that are similar in analytical behavior to the compounds of interest, and not expected to be found in the samples otherwise.

Whichever internal standards are employed, the analyst needs to demonstrate that the measurement of the internal standard is not affected by method analytes and surrogates or by matrix interferences. 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. The

use of MS detectors makes internal standard calibration practical because the masses of the internal standards can be resolved from those of the target compounds even when chromatographic resolution cannot be achieved.

When preparing calibration standards for use with internal standard calibration, add the same amount of the internal standard solution to each calibration standard, such that the concentration of each internal standard is constant across all of the calibration standards, whereas the concentrations of the target analytes will vary. The internal standard solution will contain one or more internal standards and the concentration of the individual internal standards may differ within the spiking solution (e.g., not all internal standards need to be at the same concentration in this solution). The mass of each internal standard added to each sample extract immediately prior to injection into the instrument or to each sample prior to purging must be the same as the mass of the internal standard in each calibration standard. The volume of the solution spiked into sample extracts should be such that minimal dilution of the extract occurs (e.g., 10 µL of solution added to a 1 mL final extract results in only a negligible 0.1% change in the final extract volume which can be ignored in the calculations).

An ideal internal standard concentration would yield a response factor of 1 for each analyte. However, this is not practical when dealing with more than a few target analytes. Therefore, as a general rule, the amount of internal standard should produce an instrument response (e.g., area counts) that is no more than 100 times that produced by the lowest concentration of the least responsive target analyte associated with the internal standard. This should result in a minimum response factor of approximately 0.01 for the least responsive target compound.

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

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

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate, in µg/L.
C_{is} = Concentration of the internal standard, in µg/L.

Note that in the equation above, RF is unitless, i.e., the units from the two area terms and the two concentration terms cancel out. Therefore, units other than µg/L may be used for the concentrations of the analyte, surrogate, and internal standard, provided that both C_s and C_{is} are expressed in the same units. The mass of the analyte and internal standard may also be used in calculating the RF value.

7.5 Calibration linearity

SW-846 chromatographic methods allow the use of both linear and non-linear models for the calibration data, as described below. Given the limitations in instrument data systems, it is likely that the analyst will have to choose one model for all analytes in a particular method. Both models can be applied to either external or internal standard calibration data.

NOTE: The option for non-linear calibration may be necessary to achieve low detection limits or to address specific instrumental techniques. However, it is not EPA's intent to allow non-linear calibration to be used to compensate for detector saturation at higher concentrations or to avoid proper instrument maintenance.

Whichever calibration model is employed, a unique analyte or surrogate concentration must fall within the calibration range. Samples with concentrations that exceed the calibration range must be diluted to fall within the range.

NOTE: The following sections describe various options for initial calibration and provide the calibration acceptance criteria used to evaluate each option. The criteria listed in these sections are designed for quantitation of trace level concentrations of the analytes of interest. If data of lesser quality will satisfy project-specific data needs, then less stringent criteria may be employed, provided that they are documented and approved in a project-specific QA project plan.

The choice of a specific calibration model should be made in one of two ways. The first is to begin with the simplest approach, the linear model through the origin, and progressing through the other options until the calibration acceptance criteria are met. The second approach is to use *a priori* knowledge of the detector response to choose the calibration model. Such knowledge may come from previous experience, knowledge of the physics of the detector, or specific manufacturer's recommendations.

7.5.1 Linear calibration using the average calibration or response factor

When calculated as described in Sec. 7.4, both calibration factors and response factors are a measure of the slope of the calibration relationship and assume that the curve passes through the origin. Under ideal conditions, the factors will not vary with the concentration of the standard that is injected into the instrument. In practice, some variation is to be expected. However, when the variation, measured as the relative standard deviation (RSD), is less than or equal to 20%, the use of the linear model is generally appropriate, and the calibration curve can be assumed to be linear and to pass through the origin.

NOTE: Linearity through zero is a statistical assumption and not a rationale for reporting results below the calibration range demonstrated by the analysis of the standards

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

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

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

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

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

If the RSD of the calibration or response factors is less than or equal to 20% over the calibration range, then linearity through the origin may be assumed, and the average calibration or response factor may be used to determine sample concentrations.

7.5.1.1 Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed the 20% acceptance limit for the RSD for a given calibration. In those instances, the following steps are recommended, but not required.

The first step is generally to check the instrument operating conditions. The suggested maintenance procedures in Sec. 7.11 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 RSD for any analyte is greater than 20%, the analyst may wish to review the results (area counts, calibration or response factors, and RSD) for those analytes to ensure that the problem is not associated with just one of the five initial calibration standards. If the problem appears to be associated with a single standard, that one standard may be reanalyzed and the RSD recalculated. Replacing the standard may be necessary in some cases.

A third alternative 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. The 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 will affect the overall sensitivity of the method. Consider the regulatory limits or action levels associated with the target analytes when adjusting the lower end of the range.

NOTE: As noted in Sec. 7.4.1.2, the method quantitation limit 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 method quantitation limit. When the purpose of the analysis is to demonstrate compliance with a specific regulatory limit or action level, the analyst must ensure that the method quantitation limit is at least as low as the regulatory limit or action level.

7.5.1.2 In those instances where the RSD for one or more analytes exceeds 20%, the initial calibration may still be acceptable if the following conditions are met:

7.5.1.2.1 The mean of the RSD values for all analytes in the calibration is less than or equal to 20%. The mean RSD is calculated by summing the RSD value for each analyte and dividing by the total number of analytes. If no analyte has an RSD above 20%, then the mean RSD calculation need not be performed.

7.5.1.2.2 The mean RSD criterion applies to all analytes in the standards, regardless of whether or not they are of interest for a specific project. In other words, if the target analyte is part of the calibration standard, its RSD value is included in the evaluation.

7.5.1.2.3 The data user must be provided with either a summary of the initial calibration data or a specific list of those compounds for which the RSD exceeded 20% and the results of the mean RSD calculation.

NOTE: The analyst and the data user must be aware that the use of the approach listed in Sec. 7.5.1.2.1 (i.e., the average of all RSD values $\leq$ 20%) will lead to greater uncertainty for those analytes for which the RSD is greater than 20%. The analyst and the data user should review the associated quality control results carefully, with particular attention to the matrix spike and laboratory control sample results (see Sec. 8.0), to determine if the calibration linearity poses a significant concern. If this approach is not acceptable for a particular application, then the analyst may need to employ one of the other calibration approaches (see Secs. 7.5.2 to 7.5.4) or adjust the instrument operating conditions and/or the calibration range until the RSD is $\leq$ 20%.

7.5.1.3 If all of the conditions in Sec. 7.5.1.2 are met, then the average calibration or response factor may be used to determine sample concentrations, as described in Sec. 7.10.

7.5.2 Linear calibration using a least squares regression

If the RSD of the calibration or response factors is greater than 20% over the calibration range, then linearity through the origin cannot be assumed. If this is the case, the analyst may employ a regression equation that does not pass through the origin. This approach may also be employed based on past experience or *a priori* knowledge of the instrument response. Further, at the discretion of the analyst, this approach also may be used for analytes that do meet the RSD limits in Sec. 7.5.1.

This is most easily achieved by performing a linear regression of the instrument response versus the concentration of the standards. Make certain that the instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). This is a statistical requirement and is not simply a graphical convention.

The analyst may also employ a weighted least squares regression if replicate multi-point calibrations have been performed, e.g., three 5-point curves. For all other instances, an appropriate unweighted least squares method should be used. When using a weighted linear least squares regression, the following weighting factor should be used:

$$\frac{1}{SD^2}$$

where SD is the standard deviation of the replicate results at each individual standard concentration.

The regression will produce the slope and intercept terms for a linear equation in the form:

$$y = ax + b$$

where:

y = Instrument response (peak area or height)
a = Slope of the line (also called the coefficient of x)
x = Concentration of the calibration standard
b = The intercept

The analyst should not force the line through the origin, but have the intercept calculated from the five data points. Otherwise, the problems noted with the RSD value will occur, i.e., a line through the origin will not meet the QC specifications. In addition, do not include the origin (0,0) as a sixth calibration point. The use of a linear regression may not be used as a rationale for reporting results below the calibration range demonstrated by the analysis of the standards. The regression calculation will generate a correlation coefficient (r) that is a measure of the "goodness of fit" of the regression line to the data. A value of 1.00 indicates a perfect fit. In order to be used for quantitative purposes, r must be greater than or equal to 0.99.

In calculating sample concentrations by the external standard method, the regression equation is rearranged to solve for the concentration (x), as shown below.

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

When a weighted linear least squares regression is employed, the regression equation becomes:

$$y = \frac{1}{SD^2}(ax + b)$$

which may be rearranged to solve for x, the concentration. Using internal standard quantitation, the regression equation is rearranged as shown below:

$$\frac{A_s C_{is}}{A_{is}} = aC_s + b$$

where:

A_s = Area (or height) of the peak for the target analyte in the sample
A_{is} = Area (or height) of the peak for the internal standard
C_s = Concentration of the target analyte in the calibration standard
C_{is} = Concentration of the internal standard
a = Slope of the line (also called the coefficient of C_s)
b = The intercept

In calculating sample concentrations by the internal standard method, the regression equation is rearranged to solve for the concentration of the target analyte (C_s), as shown below.

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

7.5.3 Non-linear calibration

In situations where the analyst knows that the instrument response does not follow a linear model over a sufficiently wide working range, or when the other approaches described here have not met the acceptance criteria, a non-linear calibration model may be employed.

NOTE: It is not EPA's intent to allow non-linear calibration to be used to compensate for detector saturation at higher concentrations or to avoid proper instrument maintenance. Thus, non-linear calibration should not be employed for methods or instruments previously shown to exhibit linear calibration for the analytes of interest.

When using a calibration model for quantitation, the curve must be continuous, continuously differentiable and monotonic over the calibration range. The model chosen should have no more than four parameters, i.e., if the model is polynomial, it may be no more than third order, as in the equation:

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

As noted above, the model must be continuous. A curve is continuous when it has consecutive numerical values along the function, whether increasing or decreasing, and without having breaks in the function (i.e., the pen shall never leave the paper from the minimum to the maximum). The model must also be continuously differentiable, such that all derivatives of the function are continuous functions themselves, and monotonic, such that all tangent lines of the derivative to all of the points on the calibration curve have either only positive or negative slopes.

If the model is not a polynomial, it should not include more than four parameters, i.e.,

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

where "f" indicates a function with up to four parameters.

In estimating model parameters for the calibration data, the instrumental response (y) must be treated as the dependent variable, and the calibration of the concentration standard (x) must be the independent variable. Do not force the line through the origin, i.e., do not set the intercept as 0, and do not include the origin (0,0) as a calibration point. Model estimates from the regression must be used as calculated, i.e., if the model is a polynomial, the intercept is d and may not be set to 0. Weighting in a calibration model may significantly improve its accuracy.

The statistical considerations in developing a non-linear calibration model require more data than the more traditional linear approaches described above. Whereas SW-846 methods employ five standards for a linear (first order) calibration model, a quadratic (second order) model requires six standards, and a third order polynomial requires seven standards.

Most curve fitting programs will use some form of least squares minimization to adjust the coefficients of the polynomial (a,b,c, and d, above) to obtain the polynomial that best fits the data. The "goodness of fit" of the polynomial equation is evaluated by calculating the weighted coefficient of the determination (COD).

$$COD = \frac{\sum_{i=1}^{n} (y_{obs} - \bar{y})^2 - \left(\frac{n-1}{n-p}\right) \sum_{i=1}^{n} (y_{obs} - Y_i)^2}{\sum_{i=1}^{n} (y_{obs} - \bar{y})^2}$$

where:

y_{obs} = Observed response (area) for each concentration from each initial calibration standard
$\bar{y}$ = Mean observed response from the initial calibration
Y_i = Calculated (or predicted) response at each concentration from the initial calibration(s)
n = Total number of calibration points (i.e., 6 for a quadratic model; 7 for a third order model)
p = Number of adjustable parameters in the polynomial equation (i.e., 3 for a third order; 2 for a second order polynomial)

Under ideal conditions, with a "perfect" fit of the model to the data, the coefficient of the determination will equal 1.0. In order to be an acceptable non-linear calibration, the COD must be greater than or equal to 0.99.

As noted in Sec. 7.5, whichever of these options is employed, a unique analyte or surrogate concentration must fall within the calibration range. Analysts are advised to check both second and third order calibration models to ensure that this holds true (e.g., no parabolas or repeating functions in the calibration range). Samples with concentrations that exceed the calibration range must be diluted to fall within the range.

7.5.4 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 before integration or the square root of the 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:

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

7.5.4.2 The transformation model chosen should 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, nor as a "shot in the dark" to describe the calibration.

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

7.5.4.4 When the transformed data are used to develop calibration factors, those factors must meet the acceptance criteria described in Sec. 7.5.1.

7.6 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. The 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 cannot be confirmed upon further analysis.

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 that the analyst can demonstrate that they provide performance appropriate for the intended application.

NOTE: The criteria listed in Sec. 7.6 are provided for GC and HPLC procedures using non-MS or FTIR detection. Identification procedures are different for GC/MS (e.g., Methods 8260 and 8270), HPLC/MS (e.g., Methods 8321 and 8325), and GC/FT-IR (e.g., Method 8410).

7.6.1 Before establishing retention time windows, make sure that the chromatographic system is operating reliably and that the system conditions have been 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.

7.6.2 Record the retention time for each single component analyte and surrogate to three decimal places (e.g., 0.007). 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.

7.6.3 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).

7.6.4 The 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. If the default standard deviation in Sec. 7.6.3 is employed, the width of the window will be 0.03 minutes.

7.6.5 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, use the retention time of the mid-point standard of the initial calibration.

7.6.6 The laboratory must calculate absolute retention time windows for each analyte and surrogate on each chromatographic column and instrument. New retention time windows must be established when a new GC column is installed. The retention time windows should be reported with the analysis results in support of the identifications made.

7.6.7 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.

7.6.8 The 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.

7.7 Calibration verification

The calibration relationship established during the initial calibration (Sec. 7.5) 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 an SW-846 method must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed. (Some methods may specify more frequent verifications). 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 response (or calculated concentration) for an analyte is within ±15% of the response obtained during the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the CF or RF values from the initial calibration to quantitate sample results. The ±15% criterion may be superseded in certain determinative methods.

Except where the determinative method contains alternative calibration verification criteria, if the response (or calculated concentration) for any analyte varies from the mean response obtained during the initial calibration by more than ±15%, then 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 response factors calculated during continuing calibration are used to update the calibration factors or response factors used for sample quantitation. This approach, while employed in other EPA programs, amounts to a daily single-point calibration, and is not appropriate nor permitted in SW-846 chromatographic procedures for trace environmental analyses.

In keeping with the approach described for initial calibration in Sec 7.5, if the average of the responses for all analytes is within 15%, then the calibration has been verified. However, the conditions in Sec. 7.5.1.2 also apply, e.g., the average must include all analytes in the calibration, regardless of whether they are target analytes for a specific project, and the data user must be provided with the calibration verification data or a list of those analytes that exceeded the 15% limit. The effect of using the average of the response for all analytes for calibration verification will be similar to that for the initial calibration -- namely, that the quantitative results for those analytes where the difference is greater than 15% will include a greater uncertainty. The analyst and the data user should review the note in Sec. 7.5.1.2.

If the calibration does not meet the 15% limit (either on the basis of each compound or the average across all compounds), check the instrument operating conditions, and if necessary, restore them to the original settings, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not within ±15%, then a new initial calibration must be prepared.

7.7.1 Verification of linear calibrations

Calibration verification for linear calibrations involves the calculation of the percent drift or the percent difference of the instrument response between the initial calibration and each subsequent analysis of the verification standard. Use the equations below to calculate % Drift or % Difference, depending on the procedure specified in the determinative method.

$$\%\ \text{Drift} = \frac{\text{Calculated concentration} - \text{Theoretical concentration}}{\text{Theoretical concentration}} \times 100$$

where the calculated concentration is determined using the mean calibration factor or response factor from the initial calibration and the theoretical concentration is the concentration at which the standard was prepared.

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

where CF_v and RF_v are the calibration factor and the response factor (whichever applies) from the analysis of the verification standard, and $\overline{CF}$ and $\overline{RF}$ are the mean calibration factor and mean response factor from the initial calibration. Except where superseded in certain determinative methods, the % difference or % drift calculated for the calibration verification standard must be within ±15% for each analyte, or averaged across all analytes (see Sec 7.7), before any sample analyses may take place.

7.7.2 Verification of a non-linear calibration

Calibration verification of a non-linear calibration is performed using the percent drift calculation described in Sec. 7.7.1, above. Except where superseded in certain determinative methods, the % drift calculated for the calibration verification standard must be within ±15% for each analyte, or averaged across all analytes (see Sec 7.7), before any sample analyses may take place. It may also be appropriate to employ two standards at different concentrations to verify the calibration. In this instance, one standard should be near the inflection point in the curve. The choice of specific standards and concentrations is generally a method- or project-specific consideration.

7.7.3 Regardless of whether a linear or non-linear calibration model is used, if either the percent drift or percent difference criterion is not met, then no sample analyses may take place until the calibration has been verified or a new initial calibration is performed that meets the specifications in Sec. 7.5 and those in the determinative method. If the calibration cannot be verified after the analysis of a single verification standard, then adjust the instrument operating conditions and/or perform instrument maintenance (see Sec. 7.11), and analyze another aliquot of the verification standard. If the calibration cannot be verified with the second standard, then a new initial calibration must be performed.

7.7.4 All target analytes and surrogates, including those reported as non-detects, must be included in a periodic calibration for purposes of retention time confirmation and to demonstrate that calibration verification criteria are being met. The frequency of this periodic calibration is project-, method-, and analyte-specific.

7.7.5 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. The concentrations of these standards should generally reflect those observed in samples.

7.7.6 Additional analyses of the mid-point calibration verification standard during a 12-hour analytical shift are strongly recommended for methods involving external standard calibration. If the response for any analyte varies from the average initial calibration response by more than 15% in these additional determinations, corrective action (see Sec. 7.11) may be necessary to restore the system or a new calibration curve should be prepared for that compound.

The 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 sample carryover. Therefore, more frequent verification of calibration (i.e., after every 10 samples) may be necessary for the electron capture, electrochemical conductivity, photoionization, and fluorescence detectors.

Sec. 8.2.2 specifies 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 that would have to be reinjected if the QC limits are violated for the standard analysis. The results from these bracketing standards must meet the calibration verification criteria in Sec. 7.7.1 and 7.7.2 and the retention time criteria in Sec. 7.6. 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., >15%, 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.

7.7.7 Any method blanks specified 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.

7.8 Chromatographic analysis of samples

7.8.1 Introduction of sample extracts into the chromatograph varies, depending on the volatility of the compound. Volatile organics are primarily introduced by purge-and-trap techniques (Method 5030, water and Method 5035, soils). However, the 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.

7.8.1.1 Manual injection (GC)

Inject 1-5 µL of the sample extract. The use of the solvent flush technique is necessary for packed columns. Use 1-2 µL of sample extract for capillary columns.

7.8.1.2 Automated injection (GC)

Using automated injection, smaller volumes (i.e., 1 µL) may be injected, and the solvent flush technique is not necessary. Laboratories should demonstrate that the injection volume is reproducible.

7.8.1.3 Purge-and-trap

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

7.8.1.4 Manual injection (HPLC)

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

7.8.1.5 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 required, however, chromatographic performance may be adversely affected.

7.8.2 All analyses, including field samples, matrix spike samples, matrix spike duplicates, laboratory control samples, method blanks, and 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. 7.7.6 and 8.2.2, when employing external standard calibration, it is necessary that a calibration verification standard be run at the end of the sequence to bracket the sample analyses. Acceptance criteria for the initial calibration and calibration verification are described in Secs. 7.5 - 7.7.

Analysis of calibration verification standards every 10 samples is strongly recommended, especially for the highly sensitive GC and HPLC detectors that detect 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 quantitations are minimized. Samples analyzed using external standard calibration

must 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 must be taken (see Sec. 7.11) to restore the system and/or a new calibration curve must be prepared for that compound and the samples must be 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.

7.8.3 Sample concentrations are calculated by comparing sample responses with the initial calibration of the system (Sec. 7.5). 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. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, is acceptable, as long as calibration limits are not exceeded. When overlapping peaks cause errors in peak area integration, the use of peak height measurements is recommended.

7.8.4 If chromatographic peaks are masked by the presence of interferences, further sample cleanup is necessary. See Method 3600 for guidance.

7.8.5 When there are a large number of target analytes, it may be difficult to fully resolve these compounds. Examples of chromatograms for the compounds of interest are provided in many determinative methods.

7.9 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 analysis on a second column with dissimilar stationary phase, by GC/MS (full scan or SIM) or HPLC/MS (if concentration permits), HPLC/UV data at two different wavelengths, GC or HPLC data from two different detectors, or by other recognized confirmation techniques. For HPLC/UV methods, the ability to generate UV spectra with a diode array detector may provide confirmation data from a single analysis, provided that the laboratory can demonstrate this ability for typical sample extracts (not standards) by comparison to another recognized confirmation technique.

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 required for GC/MS and HPLC/MS methods.

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

When using GC/MS for confirmation, ensure that GC/MS analysis is performed on an extract at the appropriate pH for the analyte(s) being confirmed, i.e., do not look for basic analytes in an acidic extract. Certain analytes, especially pesticides, may degrade if extraction conditions were either strongly acidic and/or strongly basic.

Many chromatographic interferences result from co-elution 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 co-elution 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. 7.10.4.

7.10 Calculations

The calculation of sample results depends on the type of calibration (external or internal standard) and the calibration model employed (linear or non-linear). The following sections describe the calculations used in each instance. Specific determinative methods may contain additional information.

7.10.1 External standard calibration - linear calibration

The concentration of each analyte in the sample is determined by comparing the detector response (peak area or height) to the response for that analyte in the initial calibration. The concentration of an analyte may be calculated as follows, depending on the sample matrix:

7.10.1.1 Aqueous samples

$$\text{Concentration } (\mu g/L) = \frac{(A_s)(V_t)(D)}{(\overline{CF})(V_i)(V_s)}$$

where:

A_s = Area (or height) of the peak for the analyte in the sample.

V_t = Total volume of the concentrated extract (µL). For purge-and-trap analysis, V_t is not applicable and therefore is set at 1.

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

$\overline{CF}$ = Mean calibration factor from the initial calibration (area per ng).

V_i = Volume of the extract injected (µL). The nominal injection volume for samples and calibration standards must be the same. For purge-and-trap analysis, V_i is not applicable and therefore is set at 1. If concentration units are used in calculating the calibration factor (see Sec. 7.4.2.1), then V_i is not used in this equation.

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

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

7.10.1.2 Nonaqueous samples

$$\text{Concentration } (\mu g/kg) = \frac{(A_s)(V_t)(D)}{(\overline{CF})(V_i)(W_s)}$$

where A_s, V_t, D, $\overline{CF}$, and V_i are as described in 7.10.1.1, and

W_s = Weight of sample extracted or purged (g). Either the wet weight or dry weight may be used, depending upon the specific application of the data. If units of kilograms are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg.

For purge-and-trap analyses where a volume of methanol extract is added to organic-free reagent water and purged, V_t is the total volume of the methanol extract and V_i is the volume of methanol extract that is added to the 5 mL of organic-free reagent water.

7.10.1.3 If a linear calibration that does not pass through the origin has been employed, then the regression equation is rearranged as shown in Sec. 7.5.2, and the concentration of the analyte is calculated from the area response (y), the slope (a), and the intercept (b). When using this form of linear calibration, it is the laboratory's responsibility to ensure that the calculations take into account the volume or weight of the original sample, the dilution factor (if any), and dry weight (as applicable). One approach to this calculation is to perform the original linear regression using the concentration of the analyte in the final extract volume or the volume purged. The concentration of the analyte in the sample may then be calculated as follows:

$$C_s = \frac{(C_{ex})(V_t)}{(V_s)}$$

where:

C_s = Concentration in the sample
C_{ex} = Concentration in the final extract
V_t = Total volume of the concentrated extract
V_s = Volume of the sample extracted or purged

For solid samples, substitute the weight of the sample, W_s, for V_s.

For purge-and-trap analyses, the concentration of the analyte in the volume of the sample that is purged will be the same as in the original sample, except when dilutions are performed.

7.10.2 Internal standard calibration - linear calibration

The concentration of each analyte in the sample is calculated using the results of the initial calibration, according to one of the following sections, depending on the sample matrix:

7.10.2.1 Aqueous samples

$$\text{Concentration } (\mu g/L) = \frac{(A_s)(C_{is})(D)(V_i)}{(A_{is})(\overline{RF})(V_s)(1000)}$$

where:

A_s = Area (or height) of the peak for the analyte in the sample.

A_{is} = Area (or height) of the peak for the internal standard.

C_{is} = Concentration of the internal standard in the concentrated sample extract or volume purged in µg/L.

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

V_i = Volume of the extract injected (µL). The nominal injection volume for samples and calibration standards must be the same. For purge-and-trap analysis, V_i is not applicable and is set at 1.

$\overline{RF}$ = Mean response factor from the initial calibration. Unlike calibration factors for external standard calibration, the response factor is dimensionless (see Sec. 7.5).

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

The 1000 in the denominator represents the number of µL in 1 mL. If the injection (V_i) is expressed in mL, then the 1000 may be omitted.

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

7.10.2.2 Nonaqueous samples

$$\text{Concentration } (\mu g/kg) = \frac{(A_s)(C_{is})(D)(V_i)}{(A_{is})(\overline{RF})(W_s)(1000)}$$

where: A_s, A_{is}, C_{is}, D, and $\overline{RF}$ are the same as for aqueous samples, and

W_s = Weight of sample extracted (g). Either a dry weight or wet weight may be used, depending upon the specific application of the data. If units of kilograms are used for this term, multiply the results by 1000.

The 1000 in the denominator represents the number of µL in 1 mL. If the injection (V_i) is expressed in mL, then the 1000 may be omitted.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg.

7.10.2.3 If a linear calibration that does not pass through the origin has been employed, then the regression equation is rearranged in a fashion similar to that described in Sec. 7.10.1.3.

7.10.3 Calculations for a non-linear calibration curve

When a non-linear curve has been employed, the non-linear model is rearranged to solve for the concentration of the analyte in the extract or purge volume, and the extract concentration is converted to a sample concentration in a fashion similar to that described in Sec. 7.10.1.3.

When non-linear calibrations are employed, it is essential that the laboratory clearly document the calculation of analyte concentrations. Example calculations should be reported that clearly indicate how the instrument response (area) was converted into a sample result.

7.10.4 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. Calculate the relative percent difference (RPD) between the two results using the formula below.

$$RPD = \frac{|R_1 - R_2|}{\left(\frac{R_1 + R_2}{2}\right)} \times 100$$

where R_1 and R_2 are the results on the two columns and the vertical bars in the equation above indicate the absolute value of the difference. Therefore, the RPD is always a positive value.

7.10.4.1 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.

7.10.4.2 If no anomalies are noted, review the chromatographic conditions. If there is no evidence of chromatographic problems, report the higher result. This approach is conservative relative to protection of the environment. The data user should be advised of the disparity between the results on the two columns.

7.11 Suggested chromatographic system maintenance

Corrective measures may involve any one or more of the following remedial actions. This list is by no means comprehensive and analysts should develop expertise in troubleshooting their specific instruments and analytical procedures. The manufacturers of chromatographic instruments, detectors, columns, and accessories generally provide detailed information regarding the proper operation and limiting factors associated with their products. The importance of reading and reviewing this information cannot be over-emphasized.

7.11.1 Capillary GC columns

Routine maintenance may compel the analyst to clean and deactivate the glass injection port insert or replace it with a fresh insert that has been cleaned and deactivated with dichlorodimethylsilane. Cut off 0.5 - 1.0 m of the injector end of the column using a 90° cut. Place ferrule onto the column before cutting.

Exceptional maintenance may compel the analyst to replace gas traps and backflush the column with solvent according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the column.

7.11.2 Metal (GC) injector body

Turn off the oven and remove the analytical column when the oven has cooled. Remove the glass injection port insert. Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

Place a beaker beneath the injector port inside the GC oven. Using a wash bottle, serially rinse the entire inside of the injector port with acetone and then toluene, catching the rinsate in the beaker.

Prepare a solution of deactivating agent (dichlorodimethylsilane) following manufacturer's directions. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, serially rinse the injector body with toluene, methanol, acetone, and hexane. Reassemble the injector and replace the GC column.

7.11.3 HPLC columns

Examine the system and check for drips that are indicative of plumbing leaks. Check that tubing connectors are of the shortest possible length to minimize dead volumes and reduce band broadening. Compatible guard columns should be installed to protect analytical columns.

If degradation of resolution or changes in back pressure are observed, the first action should be to replace the guard column if one is installed. Secondly, temporarily reverse the flow through the column to dislodge contamination in the frit with the column disconnected from the detector. If this does not correct the problem, place the analytical column in a vise, remove the inlet compression fitting and examine the column.

Analysts should establish that no void volume has developed, that the column packing has not become contaminated, and that the frit is not clogged. Void volumes can be filled with compatible packing and frits replaced.

Columns must eventually be replaced as the bonding and end-capping groups used to modify the silica are lost with time. Loss of these groups will result in chromatographic tailing and changes in analyte retention times. Retention times may also change because of differences in column temperature or because the composition of the solvent gradient is not completely reproducible.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. The development of in-house QC limits for each method is encouraged, as described in Sec. 8.7. The use of instrument-specific QC limits is encouraged, provided such limits will generate data appropriate for use in the intended application. In general, the following QC requirements pertain to all the determinative methods listed in Sec. 1.1 unless superseded by specific requirements provided in the determinative method.

8.2 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 requires selection of suitable preparation and analysis methods and an experienced staff to use these methods.

8.2.1 For each 12-hour period during which analysis is performed, the performance of the entire analytical system should be checked. These checks should be part of a formal quality control program that includes the analysis of blanks, calibration standards, matrix spikes, laboratory control samples and replicate samples, although all of these checks need not be performed during each shift.

8.2.2 Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. Therefore, all sample analyses performed using external standard calibration must be bracketed with acceptable calibration verification standards.

8.2.3 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:

Do the peaks look normal (Gaussian)?
Is the response obtained comparable to the response from previous calibrations?
Do the column fittings need tightening?
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)?
Does the HPLC guard column need replacement?

8.2.4 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.

8.2.5 Recalibration of the instrument must take place when the performance changes to the point that the calibration verification acceptance criteria (Sec. 7.7) cannot be achieved. In addition, significant maintenance activities or hardware changes may also require recalibration. The sections below provide general guidance on the sorts of procedures that may or may not require recalibration.

8.2.5.1 There are various types of instrument maintenance that should <u>not</u> automatically require recalibration of the instrument. Examples include changing: septa; compressed gas cylinders; syringes; moisture, hydrocarbon, or oxygen traps; solvents in an ELCD; purge tubes; PTFE transfer lines; glow plugs; split seals; column fittings; inlets; or filaments. Other procedures include breaking off or changing a guard column or cleaning the inlet. Whenever such procedures are performed, the analyst must demonstrate that the results for a calibration verification standard meet the acceptance criteria in Sec. 7.7. before the analysis of any samples. Otherwise, recalibration is required.

8.2.5.2 In contrast to Sec. 8.2.5.1, some maintenance procedures are so likely to affect the instrument response that recalibration is <u>automatically</u> required, regardless

of the ability to meet the calibration verification acceptance criteria. These procedures include: changing, replacing, or reversing the column; recoating the bead in a detector; changing nitrogen tubes in an NPD; changing resins; changing the PID seal or lamp; changing the FID jet; changing the entrance lens, draw out lens, or repeller; cleaning the MS source; changing the electron multiplier, ion source chamber, or injector port. Whenever such procedures are performed, the analyst must perform a new initial calibration that meets the requirement using Sec 7.5. As noted in Sec. 7.6, changing or replacing the column will also require that the retention time windows be redetermined.

8.2.6 The analysis of method blanks is critical to the provision of meaningful sample results. Consult the appropriate 3500 or 5000 series method for the specifics of the preparation of method blanks. The following general guidelines apply to the interpretation of method blank results.

8.2.6.1 Method blanks should be prepared at a frequency of at least 5%, that is, one method blank for each group of up to 20 samples prepared at the same time, by the same procedures. For samples analyzed for volatiles by the purge-and-trap technique, the 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.

8.2.6.2. When samples that are extracted together are analyzed on separate instruments or on 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 were analyzed to demonstrate that the instrument is not contributing contaminants to the samples.

8.2.6.3 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.

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

8.2.6.5 As described in Chapter One, the results of the method blank should be:

8.2.6.5.1 Less than the laboratory's MDL for the analyte or less than the level of acceptable blank contamination specified in the approved quality assurance project plan.

8.2.6.5.2 Less than 5% of the regulatory limit associated with an analyte.

8.2.6.5.3 Or less than 5% of the sample result for the same analyte, whichever is greater.

8.2.6.5.4 If the method blank results do not meet the acceptance criteria above, then the laboratory should take corrective action to locate and reduce the source of the contamination and to re-extract and reanalyze any samples associated with the contaminated method blank.

8.2.6.6 The laboratory should not 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 often leads to negative sample results. If the method blank results do not meet the acceptance criteria in 8.2.6.5 and reanalysis 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.

8.2.6.7 Method blanks and/or solvent blanks may also be used to check for contamination by carryover from a high-concentration sample into subsequent samples (see Sec. 3.1). When the analysis of such blanks is not possible, such as when an unattended autosampler is employed, the analyst should review the results for at least the next two samples after the high-concentration sample. If analytes in the high-concentration sample are not present in the subsequent samples, then the lack of carryover has been demonstrated. If there is evidence that carryover may have occurred, then the samples should be reanalyzed.

8.3 Summary of required instrument QC

The following criteria primarily pertain to GC and HPLC methods with non-MS or FTIR detectors, and may be superseded by criteria specified in individual determinative methods (e.g., Methods 8021, 8260, 8270, 8321, 8325, and 8410).

8.3.1 The criteria for linearity of the initial calibration curve is an RSD of $\leq$ 20%.

8.3.2 For non-linear calibration curves, the coefficient of the determination (COD) must be greater than or equal to 0.99 (see Sec. 7.5.2).

8.3.3 Retention time (RT) windows must be established for the identification of target analytes. See Sec. 7.6 for guidance on establishing the absolute RT windows.

8.3.4 The retention times of all analytes in all verification standards must fall within the absolute RT windows. If an analyte falls outside the RT window in a calibration verification standard, new absolute RT windows must be calculated, unless instrument maintenance corrects the problem.

8.3.5 The calibration verification results must be within ± 15% of the response calculated using the initial calibration. If the limit is exceeded, a new standard curve must be prepared unless instrument maintenance corrects the problem.

8.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each combination of sample preparation and determinative methods that it utilizes, by generating data of acceptable accuracy and precision for a reference sample containing the target analytes in a clean matrix. The laboratory must also repeat this demonstration whenever new staff are trained or significant changes in instrumentation are made.

8.4.1 The 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. If prepared by the laboratory, the reference sample concentrate must be made using stock standards prepared independently from those used for calibration.

Preparation of the reference sample concentrate is dependent upon the method being evaluated. Guidance for reference sample concentrations for certain methods are listed in Sec. 8.0 of 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 that is 10 - 50 times the MDL for each analyte in that matrix.

The concentration of target analytes in the reference sample may be adjusted to more accurately reflect the concentrations that will be analyzed by the laboratory. If the concentration of an analyte is being evaluated relative to a regulatory limit or action level, see Sec. 8.5.1 for information on selecting an appropriate spiking level.

8.4.2 To evaluate the performance of the total analytical process, the reference samples must be handled in exactly the same manner as actual samples. Use a clean matrix for spiking purposes (one that does not have any target or interference compounds), e.g., organic-free reagent water for the aqueous matrix and organic-free sand or soil for the solid matrix.

8.4.3 Preparation of reference samples

8.4.3.1 Volatile organic analytes

Prepare the reference sample by adding 200 µL of the reference sample concentrate (Sec. 8.4.1) to 100 mL of organic-free reagent water. Transfer this solution immediately to a 20- or 25-mL (or four 5-mL) gas-tight syringe(s) when validating water analysis performance by Method 5030. Alternatively, the reference sample concentrate may be injected directly through the barrel of the 5- or 25-mL syringe. See Method 5000 (Sec. 8.0) for guidance on other preparative methods and matrices.

8.4.3.2 Semivolatile and nonvolatile organic analytes

Prepare the reference sample by adding 1.0 mL of the reference sample concentrate (Sec. 8.4.1) to each of four 1-L aliquots of organic-free reagent water. See Method 3500 (Sec. 8.0) for other matrices.

8.4.4 Analyze at least four replicate aliquots of the well-mixed reference samples by the same procedures used to analyze actual samples (Sec. 7.0 of 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).

8.4.5 Calculate the average recovery ($\bar{x}$) in µg/L, and the standard deviation of the recovery (s) in µg/L, for each analyte of interest using the four results.

8.4.6 Multiple-laboratory performance data are included in some determinative methods and may be used as guidance in evaluating performance in a single laboratory. Compare s and $\bar{x}$ for each analyte with the corresponding performance data for precision and accuracy given in the performance data 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 individual $\bar{x}$ value falls outside the range for accuracy, then the system performance may be unacceptable for that analyte.

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.

When one or more of the analytes fail at least one of the performance criteria, the analyst should proceed according to Sec. 8.4.6.1 or 8.4.6.2.

8.4.6.1 Locate and correct the source of the problem and repeat the test for all analytes of interest, beginning at Sec. 8.4.2.

8.4.6.2 Beginning at Sec. 8.4.2, repeat the test only for those analytes that failed 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. 8.4.2.

8.4.7 The performance data in many of the methods are based on single-laboratory performance. As with the 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.

8.4.8 Even when the determinative method contains performance data (either multiple-laboratory or single-laboratory), the development of in-house acceptance limits is strongly recommended, and may be accomplished using the general considerations described in Sec. 8.7.

8.4.9 In the absence of recommended acceptance criteria for the initial demonstration of proficiency, the laboratory should use recoveries of 70 - 130% as guidance in evaluating the results. Given that the initial demonstration is performed in a clean matrix, the average recoveries of analyte from the four replicates should generally fall within this range. In addition, since the laboratory will repeat the initial demonstration of proficiency whenever new staff are trained or significant changes in instrumentation are made, the resulting data should be used to develop in-house acceptance criteria, as described in Sec. 8.7.

8.5 Matrix spike and laboratory control samples

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this will include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair with each batch of up to 20 samples of the same matrix processed together (see Chapter One). If samples are expected to contain the target analytes of concern, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample as an alternative to the MS/MSD pair (see Sec. 8.5.3).

In the case of purge-and-trap methods, the MS/MSD, or MS and duplicate samples, should be prepared and analyzed concurrently with the samples. In the case of samples that involve an extraction procedure, the MS/MSD, or MS and duplicate samples, should be extracted with the batch of samples but may be analyzed at any time.

In addition, a Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicates 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.

The concentration of the matrix spike sample and/or the LCS should be determined as described in Secs. 8.5.1 and 8.5.2.

8.5.1 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, it is suggested that a background sample of the same matrix from the site be submitted for matrix spiking purposes to ensure that high concentrations of target analytes and/or interferences will not prevent calculation of recoveries.

8.5.2 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 sample at the same concentration as the reference sample (Sec. 8.4.1), at 20 times the estimated quantitation limit (EQL) in the matrix of interest, or at a concentration near the middle of the calibration range. It is again suggested that a background sample of the same matrix from the site be submitted as a sample for matrix spiking purposes.

8.5.3 To develop precision and accuracy data for each of 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 matrix spike and matrix spike duplicate 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.

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 specified in Sec. 8.5.1 or 8.5.2.

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 (see Secs. 8.5.1 and 8.5.2). Prepare a matrix spike duplicate sample from a third aliquot of the sample.

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.

8.5.3.1 Calculation of recovery

Accuracy 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.

$$\text{Recovery} = \%R = \frac{C_s - C_u}{C_n} \times 100$$

where:

C_s = Measured concentration of the spiked sample aliquot
C_u = Measured concentration of the unspiked sample aliquot (use 0 for the LCS)
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS)

8.5.3.2 Calculation of precision

Precision is estimated from the relative percent difference (RPD) of the concentrations (*not* the recoveries) measured for matrix spike/matrix spike duplicate pairs, or for duplicate analyses of unspiked samples. Calculate the RPD according to the formula below.

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

where:

C_1 = Measured concentration of the first sample aliquot
C_2 = Measured concentration of the second sample aliquot

8.5.4 Recommended QC acceptance criteria for matrix spike samples and LCS

It is necessary for the laboratory to develop single-laboratory performance data for accuracy and precision in the matrices of interest (see Sec. 8.7). 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 (see Sec. 8.7). Where in-house limits have been developed for matrix spike recoveries, the LCS results should fall within those limits, as the LCS is prepared in a clean matrix.

Even where the determinative methods provide performance criteria for matrix spikes and LCS, it is necessary for laboratories to develop in-house performance criteria and compare them to those in the methods. The development of in-house performance criteria is discussed in Sec. 8.7.

As a general rule, the recoveries of most compounds spiked into samples should fall within the range of 70 - 130%, and this range should be used as a guide in evaluating in-house performance. However, as described in Sec. 8.5.4.1, matrix spike recoveries and LCS recoveries may be affected by the spike-to-background ratio.

Where methods do contain performance data for the matrix of interest, use Secs. 8.5.4.1 and 8.5.4.2 as guidance in evaluating data generated by the laboratory.

8.5.4.1 When multiple-laboratory performance data for the matrix of interest are provided in the determinative method, compare the percent recovery (%R) for each analyte in a water sample with the performance data. Given that such performance criteria were developed from multi-laboratory data, they should be met in almost all laboratories. See Sec. 8.7.10 for more information on comparisons between limits. The performance data include an allowance for error in measurement of both the background and spike concentrations, and assume a spike-to-background ratio of 5:1. If spiking was performed at a concentration lower than that used for the reference sample (Sec. 8.4), the analyst may use either the performance data presented in the tables, or laboratory-generated QC acceptance criteria calculated for the specific spike concentration, provided that they meet the project-specific data quality objectives.

8.5.4.2 When the sample was spiked at a spike-to-background ratio other than 5:1, the laboratory should calculate acceptance criteria for the recovery of an analyte. Some determinative methods contain a table entitled "Method Accuracy and Precision as a Function of Concentration" which gives equations for calculating accuracy and precision as a function of the spiking concentration. These equations may be used as guidance in establishing the acceptance criteria for matrix spike samples.

The equations are the result of linear regression analyses of the performance data from a multiple-laboratory study. The equations are of the form:

$$\text{Accuracy} = x' = (a)C + b$$

where a is a number less than 1.0, b is a value greater than 0.0, and C is the test concentration (or true value).

Performance criteria for accuracy may be calculated from these equations by substituting the spiking concentration used by the laboratory in place of "C," and using the values of a and b given in the table for each analyte.

Performance criteria for precision are calculated in a similar fashion, using the a and b values for precision given in the table for each analyte. Precision may be calculated as single analyst precision, or overall precision, using the appropriate equations from the table. An acceptable performance range may be calculated for each analyte as:

$$\text{Acceptance range } (\mu g/L) = \text{Accuracy} \pm (2.44)\text{Precision}$$

8.5.5 Also compare the recovery data from the matrix spike with the LCS data (use the average recovery if a matrix spike and matrix spike duplicate were analyzed). If any individual percent recovery in the matrix spike (or matrix spike duplicate) 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 matrix spike data exceed the limits. The surrogate recovery data (Sec. 8.6) should also be used to evaluate the data. Recoveries of both matrix spike compounds and surrogates that are outside of the acceptance limits suggest more pervasive analytical problems then problems with the recoveries of either matrix spikes or surrogates alone.

8.6 Surrogate recoveries

8.6.1 It is necessary that the laboratory evaluate surrogate recovery data from individual samples versus surrogate recovery limits developed in the laboratory. The general considerations for developing in-house acceptance criteria for surrogate recoveries are described in Sec. 8.7.

8.6.2 Surrogate recovery is calculated as:

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

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

8.6.2.1 Check to be sure that there are no errors in the calculations, surrogate solutions or internal standards. If errors are found, recalculate the data accordingly. Examine chromatograms for interfering peaks and integrated peak areas.

8.6.2.2 Check instrument performance. If an instrument performance problem is identified, correct the problem and re-analyze the extract (or re-analyze the sample for volatiles).

8.6.2.3 Some samples may require dilution in order to bring one or more target analytes within the calibration range or to overcome significant interferences with some analytes. This may result in the dilution of the surrogate responses to the point that the recoveries can not be measured. If the 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 were within the QC limits, and no further action is required. However, the results of both the diluted and undiluted (or less-diluted) analyses should be provided to the data user.

8.6.2.4 If no instrument problem is found, the sample should be re-extracted and re-analyzed (or re-analyze the sample for volatiles).

8.6.2.5 If, upon re-analysis (in either 8.6.2.2 or 8.6.2.4), the recovery is again not within limits, report the data as an "estimated concentration." If the recovery is within the limits in the re-analysis, provide the re-analysis data to the data user. If the holding time for the method has expired prior to the re-analysis, provide both the original and re-analysis results to the data user, and note the holding time problem.

8.7 Generating performance criteria for matrix spike recoveries, surrogate recoveries, initial demonstration of proficiency, and laboratory control sample recoveries

It is essential that laboratories calculate in-house performance criteria for matrix spike recoveries and surrogate recoveries. It may also be useful to calculate such in-house criteria for laboratory control sample (LCS) recoveries and for the initial demonstration of proficiency when experience indicates that the criteria recommended in specific methods are frequently missed for some analytes or matrices. The development of in-house performance criteria and the use of control charts or similar procedures to track laboratory performance cannot be over-emphasized. Many data systems and commercially-available software packages support the use of control charts.

The procedures for the calculation of in-house performance criteria for matrix spike recovery and surrogate recovery are provided below. These procedures may also be applied to the development of in-house criteria for the initial demonstration of proficiency and for LCS recoveries.

8.7.1 For each matrix spike sample analyzed, calculate the percent recovery of each matrix spike compound added to the sample, in a fashion similar to that described in Sec. 8.5.3.3. For each field sample, calculate the percent recovery of each surrogate as described in Sec. 8.6.

8.7.2 Calculate the average percent recovery (p) and the standard deviation (s) for each of the matrix spike compounds after analysis of 15-20 matrix spike samples of the same matrix, using the equations in Sec. 7.5.1, as guidance. Calculate the average percent recovery (p) and the standard deviation (s) for each of the surrogates after analysis of 15-20 field samples of the same matrix, in a similar fashion.

8.7.3 After the analysis of 15-20 matrix spike samples of a particular matrix (or matrix spike limits) or 15-20 field samples (for surrogate limits), calculate upper and lower control limit for each matrix spike or surrogate compound:

Upper control limit = p + 3s
Lower control limit = p - 3s

Calculate warning limits as:

Upper warning limit = p + 2s
Lower warning limit = p - 2s

For laboratories employing statistical software to determine these limits, the control limits approximate a 99% confidence interval around the mean recovery, while the warning limits approximate a 95% confidence interval.

8.7.4 Any matrix spike, surrogate, or LCS results outside of the control limits require evaluation by the laboratory. Such actions should begin with a comparison of the results from the samples or matrix spike samples with the LCS results. If the recoveries of the analytes in the LCS are outside of the control limits, then the problem may lie with the application of the extraction and/or cleanup procedures applied to the sample matrix or with the chromatographic procedures. Once the problem has been identified and addressed, corrective action may include the reanalysis of samples, or the extraction and analysis of new sample aliquots, including new matrix spike samples and LCS.

When the LCS results are within the control limits, the problem may either be related to the specific sample matrix or to an inappropriate choice of extraction, cleanup, and determinative methods. If the results are to be used for regulatory compliance monitoring, then the analyst must take steps to demonstrate that the analytes of concern can be determined in the sample matrix at the levels of interest.

The laboratory may use the warning limits to guide internal evaluations of method performance, track the performance of individual analysts, and monitor the effects of changes to the analytical procedures. Repeated results outside of the warning limits should lead to further evaluation.

8.7.5 Once established, control limits and warning limits for matrix spike compounds should be reviewed after every 10-20 matrix spike samples of the same matrix, and updated at least semi-annually. Control limits and warning limits for surrogates should be reviewed after every 20-30 field samples of the same matrix, and should be updated at least semi-annually. The laboratory should track trends in both performance and in the control limits themselves. The control and warning limits used to evaluate the sample results should be those in place at the time that the sample was analyzed. Once limits are updated, those limits should apply to all subsequent analyses of new samples.

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

8.7.7 Results used to develop acceptance criteria should meet all other QC criteria associated with the determinative method. For instance, matrix spike recoveries from a GC/MS procedure should be generated from samples analyzed after a valid GC/MS tune and a valid initial calibration that includes the matrix spike compounds. Another example is that analytes in GC or HPLC methods must fall within the established retention time windows in order to be used to develop acceptance criteria.

8.7.8 Laboratories are advised to consider the effects of the spiking concentration on matrix spike performance criteria, and to avoid censoring of data. As noted in Sec. 8.5.4, the acceptance criteria for matrix spike recovery and precision are often a function of the spike concentration used. Therefore, use caution when pooling matrix spike/matrix spike duplicate data for use in establishing acceptance criteria. Not only should the results all be from the same (or very similar) matrix, but the spiking levels should also be approximately the same (within a factor of 2).

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

8.7.9 Another common error in developing acceptance criteria is to discard data that do not meet a preconceived notion of acceptable performance. This results in a censored data set, which, when used to develop acceptance criteria, will lead to unrealistically narrow criteria. Remember that for a 95% confidence interval, 1 out of every 20 observations likely will still fall outside the limits.

While professional judgement is important in evaluating data to be used to develop acceptance criteria, do not discard specific results simply because they do not meet one's expectations. Rather, employ a statistical test for outlier values, or at least calculate the acceptance limits both with and without the results that are considered suspect and observe the effect of deleting suspect data.

8.7.10 In-house QC limits must be examined for reasonableness. It is not EPA's intent to legitimize poor recoveries that are due to the incorrect choice of methods or spiking levels. In-house limits also should be compared with the objectives 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 the analysis of samples near but below a regulatory limit, because of the potential high bias.

It may be useful to compare QC limits generated in the laboratory to the performance data that may be listed in specific determinative methods. However, the analyst must be aware that performance data generated from multiple-laboratory data tend to be significantly wider than those generated from single-laboratory data. In addition, comparisons between in-house limits and those from other sources should generally focus more on the accuracy (recovery) limits of single analyses rather than the precision limits. For example, a mean recovery closer to 100% is generally preferred, even if the ±3 standard deviation range is slightly wider, because those limits indicate that the result is likely closer to the "true value." In contrast, the precision range provides an indication of the results that might be expected from repeated analyses of the same sample.

8.8 It is recommended that the laboratory adopt additional quality assurance practices for use with these methods. The specific practices that are most productive depend upon the needs of the laboratory, the nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess the precision of the environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element 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.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in the SW-846 analytical methods generally were obtained using organic-free reagent water. Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects. See Chapter One for more guidance on determination of laboratory-specific MDLs.

9.2 Refer to the determinative methods for method performance information.

10.0 REFERENCES

For further information regarding these methods, review Methods 3500, 3600, 5000, and Chapter One.