Laboratory Procedure Manual

Analyte: **Volatile Organic Compounds (VOCs)**

Matrix: **Whole Blood**

Method: **Solid Phase Microextraction Benchtop GCMS**

Method No: **13-OD**

Revised:

as performed by:

Emergency Response & Air Toxicants Branch
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Important Information for Users

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999-2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
Lab 04	LBXV4C	Tetrachloroethene(ng/mL)
	LBXVBF	Bromoform(pg.mL)
	LBXVBM	Bromodichloromethane(pg/mL)
	LBXVBZ	Benzene(ng/mL)
	LBXVCF	Chloroform(pg/mL)
	LBXVCM	Dibromochloromethane(pg/mL)
	LBXVCT	Carbon Tetrachloride(ng/mL)
	LBXVDB	1,4-Dichlorobenzene(ng/mL)
	LBXVEB	Ethylbenzene(ng/mL)
	LBXVME	MTBE(pg/mL)
	LBXVOX	o-Xylene(ng/mL)
	LBXVST	Styrene(ng/mL)
	LBXVTC	Trichloroethene(ng/mL)
	LBXV3A	1,1,1-Trichloroethene(ng/mL)
	LBXVTO	Toluene(ng/mL)
	LBXVXY	m-/p-Xylene(ng/mL)

1. Clinical Relevance and Summary of Test Principle

Volatile organic compounds (VOCs) are measured in especially collected whole blood samples by headspace solid phase microextraction/gas chromatography/isotope dilution mass spectrometry based on the method described by Cardinali, et al. (1). The analytes are in equilibrium between the whole blood matrix and the headspace above the sample. A solid-phase microextraction fiber is inserted into the headspace and the VOCs partition into the phase on the outside of the fiber shaft. This fiber is then inserted into the heated

GC inlet where the VOCs rapidly desorb due to the increased temperature. Extracted VOCs are focused at the head of the GC column using a cryogenic trap. Analytes are separated on a capillary column designed for VOC analyses and quantified using SIMS MS (unit mass resolution). Comparison of relative response factors with known standard concentrations yields individual analyte concentrations. The method is applicable to the determination of 38 VOCs in 3 mL blood with detection limits in the low parts per trillion ranges. Because non-occupationally exposed individuals have blood VOC concentrations in this range, the method is applicable for determining these quantities and investigating cases of low-level exposure to VOCs.

2. Safety Precautions

A. Reagent toxicity or carcinogenicity

ALL OF THE COMPOUNDS USED IN THIS STUDY ARE HAZARDOUS CHEMICALS! Use a high draft fume hood and lower all the sashes to recommended operating height when working with neat (undiluted) materials or highly concentrated solutions since a number of these compounds are toxic. Wear vinyl or nitrile gloves when handling hazardous chemicals to prevent absorption through the skin. Some of the compounds used in this study are known or suspected carcinogens, mutagens and/or teratogens.

B. Radioactive hazards

None.

C. Microbiological hazards

Follow Universal Precautions. Because of the possibility of exposure to various microbiological hazards, appropriate measures should be taken to avoid any direct contact with the blood specimens. A Hepatitis B vaccination series is recommended for health care and laboratory workers who are exposed to human fluids and tissues.

D. Mechanical hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Laboratorians should read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of the gas chromatograph or mass spectrometer unless all power to the instrument is off. Generally, mechanical and electronic maintenance and repair should only be performed by qualified technicians. The autosampler and the mass spectrometer contain a number of areas which are hot enough to cause burns. Precautions should be used when working in these areas.

E. Protective equipment

Standard safety precautions should be followed when performing this procedure, including the use of a lab coat/disposable gown, safety glasses, appropriate gloves,

and chemical fume hood. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

F. Training

Formal training in the use of the gas chromatograph and mass spectrometer is necessary. Users are required to read the operation manuals and should demonstrate safe techniques in performing the method.

G. Personal hygiene

Follow Universal Precautions. Care should be taken when handling chemicals or any biological specimen. Routine use of gloves and proper hand washing should be practiced. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

H. Disposal of wastes

Waste materials must be disposed of in compliance with CDC laboratory, federal, state, and local regulations. Solvents and reagents should always be disposed of in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood. Disposable plastic, glass, and paper (e.g. pipette tips, vacutainers, gloves, etc.) that contact blood are placed in a biohazard autoclave bag. The biohazard autoclave bags should be kept in appropriate containers until sealed and autoclaved. Wipe down all surfaces with fresh 70% ethanol solution when work is finished. Disposable needles used to remove blood from syringes should be placed immediately into a sharps container and autoclaved when the sharps container becomes full. All syringes and other non-disposable glassware that contact blood should be decontaminated with a freshly prepared bleach solution (a 10% dilution of commercial sodium hypochlorite (bleach) or equivalent) before re-use or disposal. Commercial sodium hypochlorite solutions contain significant amounts of chloroform and bromodichloromethane that can contaminate samples; routine disinfection with bleach should therefore be isolated from preparatory areas and VOC blood samples.

Observe Universal Precautions. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.

All syringes and other non-disposable glassware that contact blood should be decontaminated with a freshly prepared bleach solution (a 10% dilution of commercial sodium hypochlorite (bleach) or equivalent) before re-use or disposal.

3. Computerization; Data-System Management

A. Software and knowledge requirements

This method has been validated using the solid phase microextraction technique coupled with a gas chromatography and a quadrupole mass spectrometer run with the ChemStation software. Data are converted from the ChemStation software format to ThermoFinnigan's Xcaliber format for review. The reviewed data is then converted to Microsoft Excel files and entered into the ATLIS relational database. Knowledge of and experience with these software packages (or their equivalent) are required to utilize and maintain the data management structure.

B. Sample information

Information pertaining to particular specimens is entered into the database either manually or electronically transferred. The result file is transferred electronically into the database. No personal identifiers are used, and all samples are referenced to a blind coded sample identifier.

C. Data maintenance

Integrity of specimen and analytical data generated by this method is maintained by visual evaluation of all relevant peak integration events, proofreading all transcribed data, storage of data in multiple computer systems, and redundant data archiving. Original data files contain traceable header information (i.e., date, analytical run number, sample type and sample identification) and are stored in duplicate on two separate recordable compact disks. Once a compact disk (CD) is filled and verified for integrity, both copies are permanently archived. Thus, there are always two copies of all data for access. One CD is stored in an office adjacent to the laboratory and the other is stored in a separate facility. Data is transferred directly by Ethernet connection to a separate PC data station for data analysis. Raw data is archived in the original ChemStation format (*.D) as well as Xcalibur format (*.RAW). The raw data are also archived on the shared network drive along with relevant meta-data including peak integrations, calibration curves, blanks, and isotope corrections. Processed results files are transferred electronically into the local area network (LAN) and stored in a shared directory. Processed data is loaded into the Microsoft Access database system using an automated data import module. Database files are backed up daily onto recordable CD and tape media.

Routine backup procedures include: 1) immediate backup of analysis data either to CD or another computer; 2) daily backup of Access database files; 3) weekly backup of database files onto CD. A separate tape database backup is stored off site. Either the supervisor or the local area network manager should be contacted for emergency assistance.

Documentation for system maintenance is contained in bound instrument-specific laboratory notebooks.

D. Information security

Information security is managed at multiple levels. The information management systems that contain the final reportable results are restricted through user ID and password security access. The computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided at multiple levels through restricted access to the individual laboratories, buildings, and site. Confidentiality of results is protected by referencing results to blind coded sample IDs (no names or personal identifiers).

4. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection

A. Special instructions

No special instructions such as fasting or special diets are required.

B. Sample collection

Isopropyl alcohol, which may be used to disinfect the venipuncture site, can contaminate the collected sample and cause nonspecific interferences in the analytical measurement process. Isopropyl alcohol contamination can be easily prevented by swabbing the venipuncture site with a dry gauze bandage and allowing the site to dry for 5 to 10 sec after wiping with isopropyl alcohol.

The specimen type is whole blood collected in specially prepared, glass vacutainers containing potassium oxalate and sodium fluoride. Additional information on preparation of these vacutainers can be found in Section 6.d.

C. Sample handling

The CDC-prepared vacutainers contain milligram quantities of potassium oxalate and sodium fluoride. These chemicals function to inhibit metabolism and prevent coagulation. Metabolic inhibition increases sample shelf life by minimizing metabolic impact on blood VOC levels during storage. This mixture's ability to prevent clotting of blood is not as great as many other anticoagulants. Thus, once samples have been collected, they must be mixed thoroughly to allow the complete distribution of the anticoagulant. If a blood mixer is available, samples should be placed on this mixer for at least 3 min. If a mixer is not available, the blood can be mixed by hand by inverting the tube 30 times. Because blood is perishable and VOCs are highly volatile, care must be taken to insure that samples are kept at refrigerator temperatures (i.e., 2-6ºC) during storage and shipment. All samples should be placed on wet ice or into a refrigerator within 30 min of sample collection. In addition, samples should be shipped with enough wet ice or equivalent cooling material to insure that the samples will remain cool (but not frozen) throughout the shipment process. Samples should be

shipped to ensure that they will arrive at CDC on normal business days to guarantee their proper processing upon arrival. Samples should not be frozen or stored at freezer temperatures at any time during sample collection and shipment. Samples should be shipped within 1 to 2 days of collection so that they can be analyzed within 2 to 3 weeks of collection.

Specimen stability has been demonstrated for analytes measured by this method for 16 weeks at refrigerated temperatures (2-6ºC). Note that blood samples change with time of refrigerated storage so that the blood is often clotted and therefore difficult to handle after 10 weeks of storage. Because these are whole blood samples, longer storage results in samples which are harder to manipulate and produce additional analytical problems. Thus, even though analytical results may not change over this time, samples may be less amenable to analysis. Volatile organic compounds occur naturally in the body, and metabolism may alter their concentration with storage.

Whole blood samples for VOC measurement should be stored at 2-6ºC. This prevents blood cell rupture which would occur during freezing. In addition, freezing of blood can lead to breakage of vacutainers and loss of sample in some cases. Since VOCs are lost whenever the containers in which they are contained are opened, it is not appropriate to transfer the blood samples to another container which would be more resistant to breaking.

D. Sample quantity

The optimal amount of specimen required for analysis is 10 mL; the minimum amount is 3 mL.

E. Unacceptable specimens

The criteria for unacceptable specimen are a low volume (< 3 mL), failure to maintain sample temperature between 2ºC and 6ºC for an extended period of time, suspected contamination, use of an untreated vacutainer, and significant clotting of the specimen. Clotting can occur due to the failure to properly mix the sample as described above.

Failure to obtain adequate sample volume is obvious when the samples are received. Visual inspection of the vacutainer reveals if the estimated blood volume is less than the required 3 mL. Maintenance of temperature during shipment is verified by examining the shipment temperature upon receipt. Clotting is indicated by failure of the sample to flow when the vacutainer tube is inverted. A description of reasons for each rejected sample is recorded in the Access database as the samples are logged into the lab.

5. Procedures for Microscopic Examinations; Criteria for Rejecting Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagents and sources

1. Solvents

Solvents used during the development, validation, and application of this method are listed below.

HPLC grade acetone is used for primary dilution of neat native standards and labeled analogs for improved solubility of nonpolar compounds. An acceptable HPLC grade acetone is sold by Sigma-Aldrich Co. (Milwaukee, WI). Other sources of HPLC grade acetone must first be shown to not contribute contaminants to the analytical measurement before use.

Purge and Trap grade methanol is required for secondary dilutions of native standards and labeled analogs. An acceptable purge and trap grade methanol is produced by Burdick and Jackson, and is acquired through KSE (Durham, NC). Other sources of purge and trap grade methanol must first be shown to not contribute contaminants to the analytical measurement before use.

HPLC grade water is primarily used for final dilutions. An acceptable purity is produced by Baker-Mallinckrodt and can be acquired from Lab Depot Supply Co. (Alpharetta, GA). However, variability in the contaminant levels in this product requires the testing of product lots. Once an acceptable lot has been found, a 1-year supply of water is purchased to insure an adequate supply. This water is further processed by helium sparging and distillation to further reduce VOCs before use. Directions for this procedure are given in Section 6.e and are based on previously published techniques for removing residual VOCs from reagent water (4).

2. Calibration and Control Materials

Material used for preparation of calibration standards and Quality Control materials are listed in Table 1. Material used for labeled internal standards are listed in Table 2. All chemicals are used without further purification unless otherwise noted. Materials procured from other sources should meet or exceed these listed requirements.

Table 1. Reagents for Calibration and Control Materials

Compound	Formula	Acceptable Grade	Safety	Source
Furan	C_4H_4O	99%	a,b,d,e	h
1,1-Dichloroethylene	$CH_2=CCl_2$	99%	c,d	h
Acrylonitrile	$N\equiv CCHCH_2$	99%	a,b,d,g,e	h
Methylene Chloride	CH_2Cl_2	99%	b,e	h
trans -1,2-Dichloroethylene	$CHCl=CHCl$	98%	d,f	h
tert -Butyl Methyl Ether	$(CH_3)_3COCH_3$	99%	d,e	i
1,1-Dichloroethane	CH_3CHCl_2	98%	a,b	i
n -Hexane	$CH_3CH_2CH_2CH_2CH_2CH_3$	99%	b,d,e	h
cis -1,2-Dichloroethylene	$CHCl=CHCl$	97%	d,f	h
Chloroform	$CHCl_3$	99%	a,b	h
1,2-Dichloroethane	CH_2ClCH_2Cl	99%	a,d	h
1,1,1-Trichloroethane	CH_3CCl_3	97%	a,b	h
Carbon Tetrachloride	CCl_4	99%	a,b	h
Benzene	C_6H_6	99%	a,d	h
Dibromomethane	CH_2Br_2	99%	b	h
1,2-Dichloropropane	$CH_3CHClCH_2Cl$	99%	d,e	h
Trichloroethylene	$CHCl=CCl_2$	99%	a,g	h
Bromodichloromethane	$CHCl_2Br$	98%	a,b	h
2,5-Dimethylfuran	$C_6H_{12}O$	99%	b,d	h
1,1,2-Trichloroethane	$CH_2ClCHCl_2$	98%	a,b	h
Toluene	$C_6H_5CH_3$	99%	b,d	h
Dibromochloromethane	$CHClBr_2$	98%	e	h
1,2-Dibromoethane	$Br^{13}CH_2{}^{13}CH_2Br$	99%	a,b,e	h
Tetrachloroethylene	$CCl_2=CCl_2$	99%	a,g	h
Chlorobenzene	C_6H_5Cl	99%	d,e	h
Ethylbenzene	$C_6H_5CH_2CH_3$	99%	d,e	h
m /*p* -Xylene	$C_6H_4(CH_3)_2$	99%	d,e	h
Bromoform	$CHBr_3$	99%	a,c	h
Styrene	$C_6H_5CH=CH_2$	99%	a,d	h
1,1,2,2-Tetrachloroethane	$CHCl_2CHCl_2$	99%	a,c	h
o -Xylene	$C_6H_4(CH_3)_2$	98%	d,e	h
1,3-Dichlorobenzene	$C_6H_4Cl_2$	98%	b,e	h
1,4-Dichlorobenzene	$C_6H_4Cl_2$	99%	a,b	h
1,2-Dichlorobenzene	$C_6H_4Cl_2$	99%	b,e	h
1,2-Dibromo-3-chloropropane	$CH_2BrCHBrCH_2Cl$	99%	b,d	h
Hexachloroethane	CCl_3CCl_3	98%	a,e	h
Nitrobenzene	$C_6H_5NO_2$	99%	b,d	h
Hexachlorobutadiene	$Cl_2C=C(Cl)C(Cl)=CCl_2$	97%	a,e	h

Key:

a - Cancer suspect agent
b - Toxic
c - Lachrymator
d - Flammable liquid
e - Irritant
f - Moisture sensitive
g - Mutagen
h - Sigma-Aldrich (Milwaukee, WI)
i - Chem Service (West Chester, PA)

Table 2. Internal Standard Compounds

Compound	Formula	Acceptable Grade	Safety	Source
Furan-D_4	C_4D_4O	98%	a,b,d,e	j
1,1-Dichloroethylene-D_2	$CD_2=CCl_2$	98%	c,d	h
Acrylonitrile-$^{13}C_3$	$N\equiv{}^{13}C{}^{13}CH{}^{13}CH_2$	99%	a,b,d,g,e	h
Methylene Chloride-$^{13}C_1$	$^{13}CH_2Cl_2$	99%	b,e	h
cis/trans -1,2-Dichloroethylene-D_2	CDCl=CDCl	97%	d,f	h
tert -Butyl Methyl Ether-D_{12}	$(CD_3)_3COCD_3$	99%	d,e	j
1,1-Dichloroethane-D_3	CD_3CHCl_2	98%	a,b	h
n -Hexane-$^{13}C_1$	$^{13}CH_3CH_2CH_2CH_2CH_2CH_3$	99%	b,d,e	j
Chloroform-$^{13}C_1$	$^{13}CHCl_3$	99%	a,b	h
1,2-Dichloroethane-D_4	CD_2ClCD_2Cl	98%	a,d	h
1,1,1-Trichloroethane-D_3	CD_3CCl_3	98%	a,b	h
Carbon Tetrachloride-$^{13}C_1$	$^{13}CCl_4$	99%	a,b	h
Benzene-$^{13}C_6$	$^{13}C_6H_6$	99%	a,d	h
Dibromomethane-D_2	CD_2Br_2	99%	b	h
1,2-Dichloropropane-D_5	$CD_3CDClCD_2Cl$	98%	d,e	h
Trichloroethylene-$^{13}C_1$	$^{13}CHCl=CCl_2$	99%	a,g	h
Bromodichloromethane-$^{13}C_1$	$^{13}CHCl_2Br$	98%	a,b	i
2,5-Dimethylfuran-$^{13}C_2$	$(^{13}CH_3)_2C_4H_6O$	99%	b,d	i
1,1,2-Trichloroethane-D_3	$CD_2ClCDCl_2$	99%	a,b	h
Toluene-$^{13}C_7$	$^{13}C_6H_5{}^{13}CH_3$	99%	b,d	j
Dibromochloromethane-$^{13}C_1$	$^{13}CHClBr_2$	98%	e	i
1,2-Dibromoethane-$^{13}C_1$	$Br^{13}CH_2{}^{13}CH_2Br$	99%	a,b,e	h
Tetrachloroethylene-$^{13}C_1$	$^{13}CCl_2=CCl_2$	99%	a,g	h
Chlorobenzene-$^{13}C_6$	$^{13}C_6H_5Cl$	99%	d,e	j
Ethylbenzene-$^{13}C_6$	$^{13}C_6H_5CH_2CH_3$	99%	d,e	h*
m -/*p* -Xylene-$^{13}C_6$	$^{13}C_6H_4(CH_3)_2$	99%	d,e	h*
Bromoform-$^{13}C_1$	$^{13}CHBr_3$	99%	a,c	h
Styrene-$^{13}C_6$	$^{13}C_6H_5CH=CH_2$	99%	a,d	j
1,1,2,2-Tetrachloroethane-D_4	$CDCl_2CDCl_2$	99%	a,c	h
o -Xylene-D_6	$C_6H_4(CD_3)_2$	98%	d,e	h*
1,3-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	98%	b,e	h
1,4-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	99%	a,b	h
1,2-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	99%	b,e	h
1,2-Dibromo-3-chloropropane-$^{13}C_3$	$^{13}CH_2Br^{13}CHBr^{13}CH_2Cl$	95%	b,d	h*
Hexachloroethane-$^{13}C_1$	$^{13}CCl_3CCl_3$	98%	a,e	h
Nitrobenzene-$^{13}C_6$	$^{13}C_6H_5NO_2$	99%	b,d	h
Hexachlorobutadiene-$^{13}C_4$	$Cl_2{}^{13}C=^{13}C(Cl)^{13}C(Cl)=^{13}CCl_2$	99%	a,e	h

Key:

a - Cancer suspect agent
b - Toxic
c - Lachrymator
d - Flammable liquid
e - Irritant
f - Moisture sensitive
g - Mutagen
h - Cambridge Isotope Laboratories (Woburn, MA) - available commercially
h* - Cambridge Isotope Laboratories (Woburn, MA) - Custom Synthesis
i - Previously available from Merck, Sharp & Dohme/Isotopes (St. Louis, MO), but currently unavailable commercially
j - Sigma-Aldrich (Milwaukee, WI)

B. Preparation of glassware

All glassware used in this study is carefully cleaned to be certain to remove possible contamination. To remove these possible analytical interferences, rinse glassware (volumetric flasks, ampoules, and storage bottles) with reagent-grade methanol, and heat at 150 ± 10°C at 10 ± 5 Torr in a vacuum oven dedicated to processing only glassware with an independent vacuum source for at least 12 hr. A dedicated vacuum oven and independent vacuum source are necessary to prevent possible cross-contamination from other materials and laboratory operations. There is the risk of changing the calibration of volumetric glassware by heating, but the error resulting from this is small compared to other sources of error in the VOC method. When the glassware is needed, cool it to room temperature under vacuum and restore pressure using nitrogen (UHP grade). Remove treated glassware from the oven and seal with polytetrafluoroethylene (PTFE) caps, when appropriate, prior to use.

C. Preparation of headspace vial septa

Headspace vial septa are nominally 20 ± 0.49-mm diameter, between 1 - 1.3 mm thick, and comprised of a PDMS-based polymer with a polytetrafluoroethylene (PTFE) barrier layer between 0.1 - 0.15 mm thick. These septa can be purchased from MicroLiter, Inc. precleaned to meet our minimal specifications equivalent to 17 hr at 110 ± 10ºC and either vacuum below 15 Torr (mmHg) or nitrogen purging above 100 mL/min. Prior to use, the septa are reprocessed for about 17 hr at 100 ± 10ºC under vacuum below 15 Torr to remove any residue or post-process contaminants from packaging, shipping and storage. After processing these septa are to remain for all applications in the oven at 70 ± 10ºC under vacuum until needed.

D. Preparation of vacutainers

The vacutainer cleaning procedure can be found in the Vacutainer Cleanup standard operating procedure located in Appendix A. Blood collection tubes (vacutainers) obtained from commercial sources contains VOC contaminants that can mask the levels of VOC analytes originally in the blood at the time of sample collection, and thus prevent accurate exposure assessment. Vacutainers are obtained commercially and specially modified by laboratory staff (DLS VOC laboratory or Battelle Volatiles laboratory) to remove measurable levels of most VOCs present. This SOP is based on our previously published research into VOC contamination from blood collection tubes (3). It is absolutely imperative that these specially treated vacutainers be used for all VOC blood collection to ensure sample validity. Following completion of vacutainer treatment and sterilization, the tubes are labeled with a new expiration date that reflects their 1-year shelf life. These tubes are supplied by DLS Lab staff for all VOC studies.

E. Preparation of Blank water

1. Apparatus

Distillation of the raw water is accomplished using a Fuchs continuous reflux apparatus which has been modified to run with helium stripping during the distillation process.

2. Procedure

a. Water distillation

Fill the 3000 mL 2-neck flask with 2500 ± 100 mL of HPLC grade water. Adjust the helium flow to produce an active flow through the sparger. Allow the helium to bubble through the raw water for at least 17 hr at 85 ± 5ºC. After 17 hr, turn on the heating mantle to bring the water to a boil. Allow the water to reflux for at least 4 hr. At the end of this period, begin collecting the finished blank water. Dispense the finished water into 100-mL glass-stoppered Pyrex bottles cleaned in accordance with Section 6.b, on glassware preparation and cap immediately. If more blank water is needed, allow the storage head to refill and repeat the process.

b. Water storage

The blank water is either used directly from the glass-stoppered Pyrex bottles or stored in 5, 10 and 20-mL flame sealable Pyrex ampoules. Water is aliquoted for storage by transfer from the Pyrex bottles using a Portapet Pipetter equipped with a 10-mL long tip serum pipette. A torch (natural gas and oxygen fuel) is used to melt the ampoule neck to produce a gas-tight seal. A hermetic seal is verified by tapping the sealed end on a paper laboratory wipe lying on a hard surface and looking for a leak. The sealed ampoules are stored in the dark at room temperature.

F. Preparation of native analytical standards

1. Procedure for handling neat compounds

Most analytes are purchased as neat liquids in flame sealed ampoules. After opening an ampoule the remaining (unused) material is discarded. A few of the most expensive analytes (custom synthesis products) are aliquotted into an individual borosilicate glass ampoules and flame sealed for future use. After transferring the compounds store the ampoules in an explosion-proof -70ºC freezer. Package all neat compound containers with aluminum foil to eliminate light exposure. Store neat standards in a dedicated chemical storage refrigerator separate from blood samples, blanks and quality control materials.

2. Procedure for filling and sealing glass ampoules

Aliquot 0.5 ± 0.049 mL of the neat standard material into a chilled 1-mL borosilicate glass ampoule. Ampoules are chilled by submerging them in liquid nitrogen between 10 and 15 sec and placing them in a pre-chilled aluminum

block tray throughout the aliquoting process. Use a glass Pasteur pipette to transfer the liquid. Before using the pipette, rinse by initially filling with the neat standard and expelling to waste. (NOTE: There may not be enough neat standard material to perform this rinse step.) Make sure the liquid is placed in the bottom of the ampoule and is not adhering to the neck of the ampoule. Otherwise, during the sealing procedure, ignition of the liquid will produce a loud pop and could shatter the ampoule. Remove the ampoule from the tray and seal using a natural gas and oxygen torch. Allow the sealed ampoule to come to room temperature then invert the vial and tap the sealed end on a laboratory wipe that is lying on a counter. If the seal does not leak, the ampoule is ready to store. If a leak does occur, do not attempt to reseal the ampoule. Dispose of it and make a new one. Repeat these steps until a sufficient number of ampoules are made. Affix plastic labels and record data, contents, and initials of those involved with preparation. Place the sealed ampoules in an appropriate holder and store in a freezer below -60ºC.

3. Transfer of liquids used in making standards

   Positive displacement pipettes are used for all transfer of liquids in the µL range. Transfers in the 5 µL to 30 µL range use a pipette with 0.1-µL increments. Transfers in the 31 µL to 100 µL range use a pipette with 0.2-µL increments. Transfers in the 101 µL to 250 µL range use a pipette with 1-µL increments. 25-mL class A volumetric flasks are used to make all standards. Standard concentrations are based on the gravimetric measure of mass transferred to the volumetric flask. Standards are prepared in methanol (purge and trap grade).

4. Final concentrations of the standards

   Standards will be prepared from low ppb to low ppt range in helium-sparged/distilled water. This involves the serial dilution of concentrated stock solutions in acetone and methanol (purge-and-trap grade), storage of a concentrated standard in flame-sealed glass ampoules, and preparation of the actual standard solution in water (helium sparged) prior to use. The resulting standards have the final concentrations given in Table 3.

Table 3. Approximate Final Standard Concentrations (ng/mL)

Compound	Standards in 25 ml water (ng/mL):						
	SS0377	SS0376	SS0375	SS0374	SS0373	SS0372	SS0371
Furan	6.88	2.18	0.688	0.218	0.069	0.022	0.007
1,1-Dichloroethylene	6.99	2.21	0.699	0.221	0.070	0.022	0.007
Acrylonitrile	93.70	29.67	9.370	2.967	0.937	0.296	0.094
Methylene Chloride	16.51	5.23	1.651	0.523	0.165	0.052	0.017
trans -1,2-Dichloroethylene	8.80	2.79	0.880	0.279	0.088	0.028	0.009
tert -Butyl Methyl Ether	9.45	2.99	0.945	0.299	0.094	0.030	0.009
1,1-Dichloroethane	4.76	1.51	0.476	0.151	0.048	0.015	0.005
n -Hexane	24.79	7.85	2.479	0.785	0.248	0.078	0.025
cis -1,2-Dichloroethylene	8.83	2.80	0.883	0.280	0.088	0.028	0.009
Chloroform	9.05	2.86	0.905	0.286	0.090	0.029	0.009
1,2-Dichloroethane	8.88	2.81	0.888	0.281	0.089	0.028	0.009
1,1,1-Trichloroethane	10.09	3.20	1.009	0.320	0.101	0.032	0.010
Carbon Tetrachloride	5.02	1.59	0.502	0.159	0.050	0.016	0.005
Benzene	12.04	3.81	1.204	0.381	0.120	0.038	0.012
Dibromomethane	18.31	5.80	1.831	0.580	0.183	0.058	0.018
1,2-Dichloropropane	7.16	2.27	0.716	0.227	0.072	0.023	0.007
Trichloroethylene	6.13	1.94	0.613	0.194	0.061	0.019	0.006
Bromodichloromethane	11.92	3.77	1.192	0.377	0.119	0.038	0.012
2,5-Dimethylfuran	11.23	3.56	1.123	0.356	0.112	0.035	0.011
1,1,2-Trichloroethane	7.27	2.30	0.727	0.230	0.073	0.023	0.007
Toluene	12.10	3.83	1.210	0.383	0.121	0.038	0.012
Dibromochloromethane	11.92	3.77	1.192	0.377	0.119	0.038	0.012
1,2-Dibromoethane	15.27	4.83	1.527	0.483	0.153	0.048	0.015
Tetrachloroethylene	9.95	3.15	0.995	0.315	0.100	0.031	0.010
Chlorobenzene	5.18	1.64	0.518	0.164	0.052	0.016	0.005
Ethylbenzene	11.98	3.79	1.198	0.379	0.120	0.038	0.012
m /*p* -Xylene	16.48	5.22	1.648	0.522	0.165	0.052	0.016
Bromoform	19.94	6.31	1.994	0.631	0.199	0.063	0.020
Styrene	25.87	8.19	2.587	0.819	0.259	0.082	0.026
1,1,2,2-Tetrachloroethane	4.96	1.57	0.496	0.157	0.050	0.016	0.005
o -Xylene	6.13	1.94	0.613	0.194	0.061	0.019	0.006
1,3-Dichlorobenzene	6.19	1.96	0.619	0.196	0.062	0.020	0.006
1,4-Dichlorobenzene	37.97	12.02	3.797	1.202	0.380	0.120	0.038
1,2-Dichlorobenzene	13.27	4.20	1.327	0.420	0.133	0.042	0.013
1,2-Dibromo-3-chloropropane	10.51	3.33	1.051	0.333	0.105	0.033	0.011
Hexachloroethane	9.48	3.00	0.948	0.300	0.095	0.030	0.009
Nitrobenzene	300.30	95.10	30.030	9.510	3.003	0.949	0.300
Hexachlorobutadiene	86.45	27.37	8.645	2.737	0.864	0.273	0.086

5. Stock solutions and concentrated standards

Primary stock solutions and intermediate standard ampoules are prepared from neat standard materials by dissolving gravimetrically confirmed amounts of standard in methanol using the following protocol. Prepare at least ten 25-mL volumetric flasks as described in Section 6.b. Label three flasks A, B, and C for the three stock solutions and label seven flasks 1 through 7 for the seven standard concentrations. Keep all flasks sealed when not directly adding standard materials. Volumes and weights of analytes necessary to prepare stock solution “A” are listed below in Table 4.

a. First add 22 ± 0.5 mL of purge and trap grade methanol to the flask labeled "A", cap, and then weigh the flask to 0.001 g. Sequentially add the neat compound to the flask listed in Table 4 starting with the least volatile compound and determine the total weight added to 0.001 g for each compound. Keep the cap tightly screwed on the flask unless volume is being added as this will minimize loss of methanol and/or analyte from evaporation.

b. Once all of the neat standards are added to the flask labeled "A", fill the flask to the 25 mark with purge and trap grade methanol. Invert this flask five times and sonicate for approximately 60 sec to ensure complete mixing. Visually inspect the flask to confirm that all solids have dissolved. Stock solution A is now prepared and ready to be further diluted for the preparation of standards.

c. Use the flask labeled "B" to dilute 500 µL of stock solution A in 25 mL of purge and trap grade methanol to yield stock solution B. Invert this flask five times and sonicate for approximately 30 sec to ensure complete mixing. Stock solution B is now prepared and ready to be further diluted for the preparation of standards.

d. Use the flask labeled "C" to dilute 500 µL of stock solution B in 25 mL of purge and trap grade methanol to give stock solution C. Invert this flask five times and sonicate for approximately 60 sec to insure complete mixing. Stock solution C is now prepared and ready to be further diluted for the preparation of standards.

e. Prepare intermediate standards 1 through 7 in their respectively labeled volumetric flask according to the dilution table shown in Table 5. For each dilution: 1) add 22 ± 0.5 mL of purge and trap grade methanol to a flask; 2) spike in the required volume of primary stock solution from the appropriate flask labeled A, B, or C; 3) dilute to 25 mL with purge and trap grade methanol; and 4) invert the capped flask five times and sonicate for approximately 30 sec.

Table 4. Primary Standard Stock Solution

Compound	Delivery vol (µl)	Density	Expected wt (mg)	Stock A (mg/ml)
Furan	50.0	0.94	46.9	1.792
1,1-Dichloroethylene	50.7	1.22	61.7	2.469
Acrylonitrile	43.0	0.8004	600.3	24.400
Methylene Chloride	87.2	1.33	116.0	4.642
trans -1,2-Dichloroethylene	48.9	1.22	59.8	2.393
tert -Methyl-Butyl Ether	182.5	0.88	160.7	6.427
1,1-Dichloroethane	27.5	1.18	32.3	1.293
n -Hexane	250.0	0.66	164.8	6.456
cis -1,2-Dichloroethylene	47.8	1.28	61.4	2.457
Chloroform	40.3	1.48	59.7	2.387
1,2-Dichloroethane	49.1	1.24	60.7	2.427
1,1,1-Trichloroethane	116.8	1.34	156.4	6.257
Carbon Tetrachloride	19.9	1.59	31.7	1.267
Benzene	90.0	0.8787	79.1	3.136
Dibromomethane	50.6	2.50	126.5	5.060
1,2-Dichloropropane	42.6	1.16	49.3	1.970
Trichloroethylene	25.2	1.46	36.8	1.471
Bromodichloromethane	39.5	1.98	78.1	3.125
2,5-dimethylfuran	80.7	0.90	72.9	2.917
1,1,2-Trichloroethane	32.2	1.44	46.4	1.857
Toluene	92.3	0.87	80.0	3.200
Dibromochloromethane	12.3	2.45	30.1	1.203
1,2-Dibromoethane	45.0	2.17	97.7	3.976
Tetrachloroethylene	89.0	1.62	144.2	5.767
Chlorobenzene	28.1	1.11	31.1	1.243
Ethylbenzene	92.1	0.86	79.2	3.167
m /*p* -Xylene	126.7	0.86	109.0	4.358
Bromoform	44.1	2.89	127.6	5.103
Styrene	195.9	0.74	145.0	5.800
1,1,2,2-Tetrachloroethane	20.2	1.60	32.3	1.293
o -Xylene	45.6	0.91	41.5	1.660
1,3-Dichlorobenzene	33.5	1.29	43.1	1.725
1,4-Dichlorobenzene	246.3	solid	246.3	9.850
1,2-Dichlorobenzene	35.7	1.31	46.6	1.865
1,2-Dibromo-3-chloropropane	31.1	2.09	65.1	2.604
Hexachloroethane	34.6	solid	34.6	1.383
Nitrobenzene	544.3	1.20	651.0	26.042
Hexachlorobutadiene	324.0	1.62	524.9	22.512

Table 5. Dilution Formulation Table for Intermediate Stock Solutions 1 - 7.

Standard Ampoule Level	Stock Solution Used	Volume Added (µL)	Final Volume (mL)
7	A	60	25
6	A	19	25
5	B	300	25
4	B	95	25
3	B	30	25
2	C	474	25
1	C	150	25

Once intermediate stock solutions have been prepared, dispense 250 µL of solution in suitably prepared 1-mL cryogenic vials as described above in the Section 6.b and flame seal as described above in Section 6.f. Make at least 75 sealed ampoules of each standard, 1 - 7. Label each ampoule with a standard batch identifier, the standard concentration level, a dash, and a sequential three digit number indicative of the preparation order of that batch. For example, "SS0371-001", where "SS037" indicates that this is the 37th set of standards produced, "1" identifies the standard concentration made (as given in column 1 in Table 5 above) and "001" is the first aliquot flame sealed in the above batch. In addition include on the label the date the vials were sealed and the initials of those involved in the preparation. The final 2 - 3 mL of the solution is more likely to change due to evaporation of analytes and/or methanol; therefore it is advisable not to aliquot until no primary stock solution remains. After leak checking the flame sealed ampoules as described in Section 6.f, place them in a 10 x 10 grid box and store in a freezer below -60ºC for later preparation of aqueous working standards.

6. Daily aqueous working standards

Daily aqueous working standards are made by diluting the above ampoules with helium sparged/distilled water. For example, standard SS0375 is created by taking 40 µL from ampoule SS0375-001 and diluting to 25 mL using helium sparged/distilled water. Following addition of internal standard, 3.0 mL of each the aqueous working standards is transferred into cleaned SPME headspace vials using a glass/Teflon multipippettor. The vials are immediately sealed with recently cleaned caps and grouped by concentration in separate 8 oz. wide mouth specimen jars to prevent cross contamination. Furthermore, the standard set is stored in a dedicated refrigerator at 4 ± 2^{o}C and subsequently analyzed as part of an analytical batch within 1 week.

G. Preparation of labeled analog solutions

1. Procedure for handling neat compounds

1, 1-Dichloroethene-D_2 is also known as vinylidene chloride-D_2 and is listed as a suspected carcinogen. Contact with the skin causes irritation and the vapors

irritate mucous membranes. The compound volatilizes readily at room temperature and is very flammable. At temperatures above 0°C and in the presence of oxygen, explosive polymerization may occur. This deuterated analog of vinylidene chloride comes from the manufacturer with hydroquinone added to stabilize the compound and prevent polymerization. For making standards, this compound is used as received from the manufacturer. An additional issue with the deuterated analog is the container in which it is shipped. The manufacturer sends this compound in a thick walled borosilicate reaction vessel of about 500-mL volume. The container is fused shut and the only way to access the contents involves scoring the container with a file and breaking it open. This presents two problems. First, the container is pressurized because of the compound's volatility and secondly, only 1 g of material occupies this large volume. To address these problems and allow transfer of this material to smaller containers which are more suitable for use in making standards, use the following approach. Place the glass shipping vessel into a bucket of ice and allow it to cool for about 2 hr. After 2 hr, a noticeable increase in the volume of liquid will be seen in the bottom of the glass container. Remove the container from the bucket and place it in a fume hood. Make sure you are wearing appropriate safety equipment before proceeding with the next step. Using a metal triangular file, score the glass container above the bulbous portion of the container at a location that will allow a good grip when snapping open the container. Because the glass is so thick, a very deep score will be needed. Thus, it is possible to break open the container during this filing operation. Be careful! After completing the scoring, place the container back in the ice and let it cool for about 10 min. After this time, remove the glass container from the bucket of ice. Under the fume hood, break open the container. The container should snap open with a minimum of force. If it does not snap easily, use the file to score it more deeply and try again. After the container is open, immediately, transfer the contents using a Pasteur pipette into, previously chilled, flame sealable glass ampoules. Place the ampoules back in the bucket of ice and remove what is needed to make standards. Seal the ampoules and store below -60°C.

Opening glass ampoules can result in broken glass that punctures fingers. While opening glass ampoules wear protective gloves or use a device that shields the hands from broken glass.

2. Procedure for filling and sealing glass ampoules

Aliquot the appropriate amount of primary or secondary analog solution into a 1-mL flame sealable borosilicate glass ampoule. Use a glass Pasteur pipette cleaned as described in Section 6.b to transfer the solution. Make sure the solution is placed in the bottom of the ampoule and is not adhering to the neck of the ampoule. Otherwise, during the sealing procedure, the ignition of the methanol will produce a loud pop and could shatter the ampoule. Place the ampoule in a pre-chilled aluminum sample tray until the liquid is cooled, but not frozen. This will require 10-15 sec. Remove the chilled ampoule from the tray and seal using a natural gas and oxygen torch. Allow the sealed ampoule to cool to room temperature then invert the vial and tap the sealed end on a hard

surface. If the seal does not leak, the ampoule is ready to store. If a leak does occur, do not attempt to reseal the ampoule. Dispose of it and make a new one. Repeat these steps until a sufficient number of ampoules are made. Place the sealed ampoules in an appropriate holder and store in a freezer at <-60°C.

3. Primary analog stock solutions

Primary analog stock solutions (i.e., L-series) are made by initial dilution of neat compound into 25 mL of purge and trap grade methanol. This provides a consistent source of these compounds for further dilutions. When mixing an L-series solution, label and fill a 25-mL volumetric flask cleaned in accordance with Section 6.b and add approximately 22 mL of fresh purge and trap grade methanol. Keep the flasks sealed when not directly adding the standard. Dilute the compound to a final volume of 25 mL with methanol according to Table 6. Once the dilution is complete invert the capped flask five times and sonicate for approximately 60 sec.

Aliquot about 0.75 mL of this solution into ampoules and flame seal as described in Section 6.f. Repeat these steps until at least 25 ampoules are prepared. Label and place the sealed ampoules in an appropriate holder and store in a freezer below -60°C.

Table 6. L-Series Primary Stock Solution Concentrations

Primary Stock	Compound	Neat delivery vol or wt	Density	Approx. conc. (g/mL)
L61	Furan-D_4	60	1.6200	2.3
L22	1,1-Dichloroethylene-D_2	50 µL	1.1560	2.4
L50	Acrylonitrile-$^{13}C_3$	100	0.9371	3.2
L11	Methylene Chloride-$^{13}C_1$	50 µL	2.8900	2.7
L6	1,2-*cis/trans* -Dichloroethylene-D_2	20 µL	1.4600	1.0
L51	*tert* -Butyl Methyl Ether-D_{12}	100 µL	1.2240	3.0
L4	1,1-Dichloroethane-D_3	50 µL	0.8787	2.4
L52	*n* -Hexane-$^{13}C_1$	100	0.8600	2.6
L12	Chloroform-$^{13}C_1$	25 µL	0.6590	1.5
L5	1,2-Dichloroethane-D_4	50 µL	2.5000	2.5
L23	1,1,1-Trichloroethane-D_3	200 µL	1.2880	10.7
L10	Carbon Tetrachloride-$^{13}C_1$	37.6 µL	0.8802	2.4
L25	Benzene-$^{13}C_6$	25 µL	1.2180	0.9
L20	Dibromomethane-D_2	20 µL	2.4500	2.0
L7	1,2-Dichloropropane-D_6	20 µL	0.9030	0.9
L16	Trichloroethylene-$^{13}C_1$	37.6 µL	solid	2.2
L18II	Bromodichloromethane-$^{13}C_1$	10 µL	0.8004	0.8
L40	2,5-Dimethylfuran-$^{13}C_2$	125 µL	1.4400	4.5
L15	1,1,2-Trichloroethane-D_3	25 µL	2.0930	1.4
L31	Toluene-$^{13}C_7$	100 µL	solid	3.5
L19II	Dibromochloromethane-$^{13}C_1$	10 µL	0.8669	1.0
L43	1,2-Dibromoethane-$^{13}C_1$	40	1.4800	3.5
L14	Tetrachloroethylene-$^{13}C_1$	25 µL	0.9100	1.6
L28	Chlorobenzene-$^{13}C_6$	50 µL	1.1760	2.2
L26	Ethylbenzene-$^{13}C_6$	25 µL	2.1720	0.9
L27	*m* /*p* -Xylene-$^{13}C_6$	50 µL	1.6200	1.7
L24II	Bromoform-$^{13}C_1$	25 µL	1.3300	2.9
L29	Styrene-$^{13}C_6$	25 µL	1.6000	0.9
L13	1,1,2,2-Tetrachloroethane-D_4	37.6 µL	1.3050	2.4
L2	*o* -Xylene-D_6	50 µL	1.1960	1.8
L41	1,3-Dichlorobenzene-$^{13}C_6$	50 µL	1.3390	2.6
L32	1,4-Dichlorobenzene-$^{13}C_6$	251.5 mg	1.5900	10.1
L3	1,2-Dichlorobenzene-$^{13}C_6$	40 µL	1.2350	2.1
L55	1,2-Dibromo-3-chloropropane-$^{13}C_3$	premixed	1.2840	1.0
L17	Hexachloroethane-$^{13}C_1$	51.8 mg	0.8600	2.1
L44	Nitrobenzene-$^{13}C_6$	50 µL	0.7400	2.4
L60	Hexachlorobutadiene-$^{13}C_4$	solid	1.1060	2.0

4. Secondary analog stock solutions

   The secondary labeled-analog stock solution is made by initial dilution of the L-series solutions into 25 mL of purge and trap grade methanol. When mixing a secondary stock solution label and fill a 25-mL volumetric flask cleaned in accordance with Section 6.b and add about 22 mL of fresh purge and trap grade methanol. Add each standard as listed in Table 7 starting from the least volatile. Keep the flask sealed when not directly adding standards. Dilute the compounds to a final volume of 25 mL with methanol. Once the dilution is complete invert the capped flask five times and sonicate for approximately 60 sec. Flame seal approximately 0.25 mL of these solutions in chilled ampoules as described above Section 6.f. Repeat these steps until at least 75 ampoules are made. Label and place the sealed ampoules in a freezer below -60^{o}C.

5. Working analog stock solutions

   Prepare the working stock solution for a given 2-wk period of analyses by adding 125 µL from a fresh ampoule of secondary stock solution to a 25-mL flask cleaned in accordance with the Section 6.b. Fill to the mark with fresh purge and trap grade methanol. Once the dilution is complete invert the capped flask five times and sonicate for about 60 sec. This solution can be reused for 2 weeks if it is sealed in a cleaned 2-mL glass sample vial, screw capped with a cleaned PTFE barrier septum and stored < -3^{o}C. To achieve the desired concentrations of internal standard in all analytical samples (i.e., blood, QCs and blank), pipette 40 µL of the working analog stock solution into each sample. Labeled standards will be prepared with the approximate final concentrations (ppb) once added to a 3-mL blood sample as given in Table 7.

Table 7. Intermediate Concentrations of Labeled Standards and Final Concentrations of Labeled Standards in Unknown Blood Samples

Compound	Primary Solution Delivery Volume (µL)	Secondary Solution Concen. (ng/mL)	Working Stock (ng/mL)	Approx. Bl Concentra (ng/mL
Furan- D_4	36	3237	25.9	0.345
1,1-Dichloroethylene-D_2	7.0	679	5.4	0.072
Acrylonitrile-$^{13}C_3$	70	8960	71.7	0.956
Methylene Chloride-$^{13}C_1$	30.0	3182	25.5	0.339
cis -/*trans* -1,2-Dichloroethylene-D_2	29.0	1166	9.3	0.124
tert -Butyl Methyl Ether-D_{12}	30.0	3554	28.4	0.379
1,1-Dichloroethane-D_3	10.0	941	7.5	0.100
n -Hexane-$^{13}C_1$	35	3690	29.5	0.394
Chloroform-$^{13}C_1$	30.0	1781	14.2	0.190
1,2-Dichloroethane-D_4	20.0	2011	16.1	0.215
1,1,1-Trichloroethane-D_3	5.0	2140	17.1	0.228
Carbon Tetrachloride-$^{13}C_1$	15.0	1434	11.5	0.153
Benzene-$^{13}C_6$	12.0	422	3.4	0.045
Dibromomethane-D_2	60.0	4792	38.3	0.511
1,2-Dichloropropane-D_5	30.0	1113	8.9	0.119
Trichloroethylene-$^{13}C_1$	8.0	705	5.6	0.075
Bromodichloromethane-$^{13}C_1$	131	4036	32.3	0.430
2,5-Dimethylfuran-$^{13}C_2$	20.0	3553	28.4	0.379
1,1,2-Trichloroethane-D_3	60.0	3460	27.7	0.369
Toluene-$^{13}C_7$	50.0	6935	55.5	0.740
Dibromochloromethane-$^{13}C_1$	60.0	2347	18.8	0.250
1,2-Dibromoethane-$^{13}C_1$	15	2086	16.7	0.222
Tetrachloroethylene-$^{13}C_1$	90.0	5843	46.7	0.623
Chlorobenzene-$^{13}C_6$	12.0	1063	8.5	0.113
Ethylbenzene-$^{13}C_6$	140.0	4850	38.8	0.517
m /*p* -Xylene-$^{13}C_6$	124.0	8558	68.5	0.913
Bromoform-$^{13}C_1$	90.0	10453	83.6	1.115
Styrene-$^{13}C_6$	250	9059	72.5	0.966
1,1,2,2-Tetrachloroethane-D_4	100	9552	76.4	1.019
o -Xylene-D_6	74.0	5211	41.7	0.556
1,3-Dichlorobenzene-$^{13}C_6$	50.0	5154	41.2	0.550
1,4-Dichlorobenzene-$^{13}C_6$	63.0	25351	202.8	2.704
1,2-Dichlorobenzene-$^{13}C_6$	38.0	3176	25.4	0.339
1,2-Dibromo-3-chloropropane-$^{13}C_3$	1850	74000	592.0	7.893
Hexachloroethane-$^{13}C_1$	100	8288	66.3	0.884
Nitrobenzene-$^{13}C_6$	1250	120500	964.0	12.853
Hexachlorobutadiene-13C4	162	12960	103.7	1.382

H. Preparation of Quality Control materials

QC Materials are prepared with the final concentrations (ppb) given in Table 8. Target concentrations and measured concentrations can vary because of significant background levels that might not be removed during the cleaning process or diffusion loss during sample preparation. The characterized mean is determined by analysis of 20 separate samples using different sample runs and instruments.

Table 8a. Experimentally Established Concentration of Precision for QC Samples

Compound	QC Concentration level	Formulation Concentration (ng/mL)	Characterized Mean (ng/mL)	% RSD among runs
Furan	High	0.218	0.145	10.36%
	Low	0.045	0.038	20.69%
1,1-Dichloroethylene	High	0.221	0.104	13.98%
	Low	0.046	0.012	24.19%
Acrylonitrile	High	2.967	0.213	60.09%
	Low	0.617	0.046	77.24%
Methylene Chloride	High	0.523	0.418	12.98%
	Low	0.109	0.096	31.21%
trans -1,2-Dichloroethylene	High	0.279	0.192	12.47%
	Low	0.058	0.029	9.90%
tert -Butyl-Methyl-Ether	High	0.299	0.295	6.86%
	Low	0.062	0.053	8.76%
1,1-Dichloroethane	High	0.151	0.114	6.58%
	Low	0.031	0.019	5.67%
n -Hexane	High	0.785	0.237	33.24%
	Low	0.163	0.102	60.73%
cis -1,2-Dichloroethylene	High	0.280	0.227	12.31%
	Low	0.058	0.043	9.09%
Chloroform	High	0.286	0.272	6.42%
	Low	0.060	0.073	5.79%
1,2-Dichloroethane	High	0.281	0.265	3.65%
	Low	0.058	0.052	3.82%
1,1,1-Trichloroethane	High	0.320	0.195	11.46%
	Low	0.066	0.035	9.89%
Carbon Tetrachloride	High	0.159	0.086	10.78%
	Low	0.033	0.015	13.73%
Benzene	High	0.381	0.365	14.16%
	Low	0.079	0.099	35.19%
Dibromomethane	High	0.580	0.557	7.20%
	Low	0.120	0.115	6.89%
1,2-Dichloropropane	High	0.227	0.195	10.10%
	Low	0.047	0.042	6.44%
Trichloroethylene	High	0.194	0.186	8.09%
	Low	0.040	0.045	13.39%
Bromodichloromethane	High	0.377	0.365	3.97%
	Low	0.078	0.082	3.44%
2,5-dimethylfuran	High	0.356	0.354	7.51%
	Low	0.074	0.068	8.33%

Table 8b. Experimentally Established Concentration of Precision for QC Samples Continued

Compound	QC Concentration level	Formulation Concentration (ng/mL)	Characterized Mean (ng/mL)	% RSD among runs
1,1,2-Trichloroethane	High	0.230	0.227	4.06%
	Low	0.048	0.050	3.82%
Toluene	High	0.383	1.280	9.67%
	Low	0.080	1.006	10.33%
Dibromochloromethane	High	0.184	0.186	2.90%
	Low	0.038	0.044	2.74%
1,2-Dibromoethane	High	0.483	0.482	3.75%
	Low	0.100	0.104	2.60%
Tetrachloroethylene	High	0.315	0.529	5.64%
	Low	0.065	0.210	6.00%
Chlorobenzene	High	0.164	0.162	4.19%
	Low	0.034	0.038	8.66%
Ethylbenzene	High	0.379	0.623	6.21%
	Low	0.079	0.311	7.30%
m-/*p*-Xylene	High	0.522	2.690	4.07%
	Low	0.108	2.008	4.95%
Bromoform	High	0.631	0.643	2.80%
	Low	0.131	0.151	3.38%
Styrene	High	0.819	0.915	7.57%
	Low	0.170	0.288	16.64%
1,1,2,2-Tetrachloroethane	High	0.157	0.155	3.29%
	Low	0.033	0.035	6.09%
o-Xylene	High	0.194	0.475	5.66%
	Low	0.040	0.317	7.18%
1,3-Dichlorobenzene	High	0.196	0.191	3.14%
	Low	0.041	0.043	6.29%
1,4-Dichlorobenzene	High	1.202	1.230	3.99%
	Low	0.250	0.290	3.62%
1,2-Dichlorobenzene	High	0.420	0.411	6.35%
	Low	0.087	0.089	8.78%
1,2-Dibromo-3-chloropropane	High	0.333	0.337	6.17%
	Low	0.069	0.065	21.13%
Hexachloroethane	High	0.300	0.305	2.98%
	Low	0.062	0.069	4.38%
Nitrobenzene	High	9.510	9.837	8.04%
	Low	1.976	2.179	8.74%
Hexachlorobutadiene	High	2.737	2.848	3.48%
	Low	0.569	0.607	5.59%

1. Preparation of bovine serum

   Fetal bovine serum (Hyclone Laboratories, Logan, UT) is cleaned by extraction using cleaned 20-mm PTFE/silicone barrier septa as the absorbent. Add about 1 L of bovine serum to a 2-L volumetric flask cleaned in accordance with Section 6.b. Add about 300 20-mL-headspace-vial septa, cleaned in accordance with Section 6.c and cooled in the vacuum oven under dry nitrogen, immediately to the bovine serum. Cap the flask with a ground glass stopper and seal the top with PTFE tape. Swirl the solution gently and place it in a dedicated refrigerator

that has been previously vented. Allow the extraction mixture is allowed to equilibrate for about 12 hr and during this time swirl the mixture gently from time to time no more than once per hour. Decant the extracted bovine serum into a cleaned 1-L volumetric flask, cap with a glass stopper, and wrap with PTFE tape. Bovine serum QC samples are prepared with the final concentrations given in Table 8.

2. Spiking serum for QC preparation

To formulate the low concentration QC solution, aliquot with a positive displacement pipette 105 µL of the *Secondary Stock Solution* SS0375 into approximately 1 L of clean bovine serum. Immediately cap and seal with PTFE tape and then gently swirl for about an hour. Place the stoppered flask on ice for about 1 hr swirling about every 15 min.

Once the low concentration QC samples have been prepared and stored, prepare the high concentration QC samples in the same manner except aliquot with a positive displacement pipette approximately 51 µL of the *Secondary Stock Solution* SS0377 into about 1 L of clean bovine serum.

3. Procedure for filling and sealing glass ampoules

Using a 10-mL serological long-tip pipette, dispense 5-8 mL of spiked serum into a cleaned 10-mL borosilicate glass ampoule that has been pre-chilled. Ampoules are chilled by submerging them in liquid nitrogen between 10 and 15 sec and placing them in a pre-chilled aluminum block tray throughout the aliquoting process. Before pipetting with a new pipette, rinse three times by initially filling with the serum and expelling to waste. Make sure the serum is placed in the bottom of the ampoule and is not adhering to the neck of the ampoule. Remove the ampoule from the tray and seal using a natural gas and oxygen torch. Allow the sealed ampoule to come to room temperature then invert the vial and tap the sealed end on a laboratory wipe that is lying on a counter. If the seal does not leak, the ampoule is ready to store. If a leak does occur, do not attempt to reseal the ampoule. Dispose of it and make a new one. Repeat these steps until a sufficient number of ampoules are made. Ensure that the ampoules are labeled with the QC formulation identifier, the ampoule series number in which it was prepared, the date on which the sample was prepared, and the initials of those involved with the preparation. Place the sealed ampoules in an appropriate holder and store in a freezer below -60^{o}C.

I. Proficiency Testing (PT) Materials

Proficiency Testing materials are prepared from neat compounds in a manner similar to standard preparation. Pooled volatile reference materials are available from Sigma-Aldrich-Supelco Chemical Company. For these tests purchase the “EPA 524 VOC mix A” and “EPA 524 Rev 4 Update mix 1” as ampoules containing 0.200 mg/mL of each of the VOC assay analytes. These solutions are combined and diluted in fresh purge and trap grade methanol to within the linear range of the VOC assay. Four PT

stock concentrations are prepared, aliquoted into ampoules, and flame sealed using the same preparation technique as described in Section 6.f. A quality control officer independent from the laboratory blind-codes the PT stock ampoules and administer the PT program. Assay performance is evaluated by blind analyses of aqueous proficiency testing samples prepared by dilution of PT stock ampoules (40 µL) with helium-sparged, distilled water.

J. Storage of standard solutions

Except while in use, all standard stock solutions, labeled analog stock solutions, and quality control materials are stored below -60°C. The working stock solutions can be stored for up to 1 week at <3°C. Once ampoules containing stock solutions have been opened, they must be used within about 5 min. After this time these materials are discarded. The working labeled analog stock solution may be preserved and used throughout a 1-week period if carefully sealed and stored at <3°C within 8 hr of initial preparation. All stock solutions are labeled to include a reference to the preparation procedure.

K. Clean-up procedure for the 5-mL Luerlock gas-tight syringe

1. Place the spent syringes into a 600-mL beaker after use.
2. Fill the beaker with a 10% bleach solution.
3. Flush each syringe three times by completely filling them with the bleach solution and expelling.
4. Fill each syringe with the bleach solution and allowed to sit for a minimum of 15 min (20 min is preferred) for decontamination.
5. Disassemble the syringes and separate the glass barrels and plungers into different 600-mL beakers.
6. Rinse the disassembled syringes thoroughly with warm tap water, filling and emptying the beakers at least three times.
7. Fill the beakers containing the disassembled syringes with HPLC grade water and sonicate in an ultrasonic bath cleaner at 30-40ºC for about 60 min.
8. Empty the HPLC rinse water from the beakers and fill with purge and trap grade methanol and sonicate at ambient temperature for about 30 min.
9. Empty the spent methanol from the beakers disposing of the methanol in a suitable waste container.
10. Rinse the beakers with ACS grade methanol, filling the beaker completely, and immediately empty the spent methanol in a suitable waste container.
11. Allow the syringes to air dry in the hood for at least 10 min.
12. Vacuum bake the syringes in their beakers by placing them into a vacuum oven at approximately 180ºC for about 24 hr under a vacuum of <15 Torr.
13. Store the syringes under vacuum at nominally 50ºC until needed for their next use.

L. Supplies

Supplies used during the development, validation, and application of this method are listed below. Supplies procured from other sources can be used but should be equivalent to the products offered by the vendors listed below.

1. Disposable Pasteur pipettes (Fisher Scientific, www1.fishersci.com)
2. Pipette bulbs (Fisher Scientific, www1.fishersci.com)
3. 5-mL, 10-mL, and 20-mL clear pre-scored ampoules (Wheaton Scientific, Millville, NJ)
4. Portapet pipetter, 10-mL volume (Fisher Scientific, www1.fishersci.com)
5. Research-grade helium gas, 99.9999% (Airgas, www.airgas.com)
6. High-density polyethylene dewar flask (Fisher Scientific, www1.fishersci.com)
7. Glassblowing kit including torch (Fisher Scientific, www1.fishersci.com)
8. Variable or fixed positive displacement micropipettors with maximum volumes that include 20-µL, 25-µL, 40-µL, 50-µL, 100-µL, and 250-µL, (VWR, West Chester, PA)
9. Glass capillaries, 20-µL, 25-µL, 40-50-µL, 100-µL, and 250-µL (VWR, West Chester, PA)
10. Pyrex volumetric flasks with screw caps, 25-mL (Fisher Scientific, www1.fishersci.com)
11. Polytetrafluoroethylene (PTFE) cap liners, No. 22, No. 33, and No. 38 (Thomas Scientific, Swedesboro, NJ)
12. Non-powdered disposable nitrile gloves (Lab Depot Inc., Alpharetta, GA)
13. Ultrasonic cleaner with heater and timer (Fisher Scientific, www1.fishersci.com)
14. Stainless steel test tube racks for 11-mm diameter tubes (VWR, West Chester, PA)
15. Serum bottles (Wheaton Scientific, Millville, NJ)
16. Septa, flat disc, red PTFE/white silicone (Integrated Liner Technologies, Albany, NJ)
17. Hand-operated crimper (Wheaton Scientific, Millville, NJ)
18. 20-mm aluminum, magnetic headspace vial (Sun-SRi, Duluth, GA)
19. Oxygen, 99.99%, 200-300 cu. ft. (local gas supply company)
20. Sterile evacuated blood collection tubes, 10-mL draw, 16 X 100, potassium oxalate, sodium fluoride (Becton/Dickinson Vacutainer Systems, Rutherford, NJ)
21. Beveled-top standard 10-mL headspace vials, (Worldwide Glass Resources, Norma, NJ)
22. 2-mL PTFE-lined screw cap vials (Agilent, www.chem.agilent.com)
23. Gastight PTFE luerlock tip syringe, 5-mL (Hamilton, Reno, NV)
24. Sharps container (Pro Tec US Clinical Products, INC., Richardson, TX)
25. Hematology mixer (Robbins Scientific, Sunnyvale, CA)
26. Sodium hypochlorite (James Austin Co., Mars, PA)
27. 150-mm flowtube for helium 0 to 100 cc/min (Alltech Associates, Inc., Deerfield, IL)
28. Adapter 1/8” to 1/8” MPT (Alltech Associates, Inc., Deerfield, IL)
29. DB-VRX Capillary Column, 0.18-mm I.D., 40-m, 1.0-µm film thickness (J&W Scientific, Folsom, CA)
30. 75-µm Carboxen/PDMS SPME fiber assembly (Supleco, www.sigmaaldrich.com/Brands/Supelco_Home.html)
31. Standard Printer paper (local office supply)

M. Equipment

Equipment used during the development, validation, and application of this method are listed below. Equipment procured from other sources can be used, but should be equivalent to the products offered by the vendors listed below.

1. Distillation Equipment (Ace Glass, Inc., Louisville, KY)
   a. Twin connecting hose adapter
   b. Column, vacuum jacketed
   c. Condenser, Allihn
   d. Head, Storage, 3000-mL
   e. Flask, two necks, 3000-mL
   f. Mantle, 3-liter
   g. Powerstat, 0 - 140 volts
   h. PTFE sleeves, 0.076-mm
   i. Adapter, vacuum short stem, 14/20
   j. PTFE sleeves, 0.13-mm, 14/20
   k. Bottle, single neck, 14/20 joint
2. Squaroid vacuum oven, 2.3 cu. ft. (Lab-line Instruments Inc., Melrose Park, IL)
3. Vacuum pump (Fisher Scientific, www1.fishersci.com)
4. Analytical Balance (Fisher Scientific, www1.fishersci.com)
5. Ultra-low temperature freezer (Fisher Scientific, www1.fishersci.com)
6. Refrigerator (Fisher Scientific, www1.fishersci.com)
7. Standard laboratory freezer (Fisher Scientific, www1.fishersci.com)
8. Sterilized hood/biological safety cabinet (A/B3, NuAir)

The following equipment has been shown to meet the requirements of the method. Substitutes must be evaluated for their ability to meet method accuracy, sensitivity, and reproducibility.

1. LEAP Combi-Pal Prep and Load system for static headspace and direct GC injections (LEAP Technologies, Carrboro, NC)
2. Gas Chromatograph (HP 6890, Agilent Technologies, www.chem.agilent.com)
3. Mass Spectrometer (5973, Agilent Technologies, www.chem.agilent.com)
4. Distilled water purifier (Barnstead, Dubuque, Iowa)
5. Access database (Microsoft, Inc., Redmond, WA)
6. Xcalibur data analysis and processing software (ThermoFinnigan, analyze.us@thermo.com)

N. Instrumentation

SPME of the headspace sample is performed using an autosampler (Combi-Pal, Leap Technologies, Carrboro NC). Samples are queued on an autosampler tray and maintained at 15 ± 0.5^{o}C until they are analyzed. During analysis the samples are transferred to an agitating incubator set to 350 rpm and 40^{o}C as the headspace is sampled with a 75-µm Carboxen-PDMS coated SPME fiber (Supelco, Bellefonte PA) for approximately 6.0 min. The SPME fiber is then immediately transferred into the GC injection port fitted with a 1-mm id glass liner and held at 250±0.5ºC. The sample is introduced onto a DB-VRX (Agilent Technologies, Wilmington DE) column (40m x

0.18mm x 1µm film) via pulsed splitless injection set at 50 psi. After 1.0 min the injection port pressure is then dropped to maintain a constant flow of 1.0 mL/min helium. In line after the injection port is a cryogenic trap (Model 961, Scientific Instrument Services, Ringoes NJ) that is maintained at approximately -100ºC for 1.0 min, then ballistically heated to approximately 225ºC (13.0ºC/sec). The GC oven temperature is ramped from 0°C (1.5 min hold) at 7°C/min to 140°C, then 40°C/min to 220°C (4.5 min hold). Quantification by a quadrupole MS (5973N, Agilent) is performed by selective ion monitoring (SIM) of each primary quantification ion, confirmation ion, and internal standard ion using at least a 15-ms dwell time for each. When required, qualitative analyses are performed using full mass scan from m/z 21 to 500. Identification of unknowns is established by GC retention time and mass spectral data. Instrumental configuration parameters for the GC/MS and CombiPAL are given in Table 9.

7. Calibration and Calibration Verification Procedures

All calibration standards are prepared in water because it proved to be difficult to consistently reduce the background VOC levels in serum or whole blood below detectable levels. Matrix spike experiments established that calibration curves in whole blood and water have the same slope. This result validates the use of water-based calibrators for quantifying VOCs in whole blood.

A. Creation of curve

1. Data Collection

A full set of 7 calibrators is analyzed with each batch of data and used for the quantification of analytes in all samples from that batch. The calibration curves are constructed for each analyte from the relative response factors for each of the 7 calibrators.

2. Calculation of curve statistics

The calibration curve is constructed from the response ratios for each analyte to its internal standard at the 7 calibration levels. Correlation coefficients should typically exceed 0.995. The slope and intercept of this curve are determined by linear least squares of data weighted 1/X using the ThermoFinnigan Xcalibur Quan software. Some compounds require correction for background and standard ion contribution to the internal standard ion response. This data transformation can be performed using the ThermoFinnigan Xcalibur Quan software.

Table 9. Gas Chromatograph and Liquid Nitrogen Cooled Cryogenic Inlet Trap Settings

GC inlet parameters	Settings
Mode:	pulsed splitless
Carrier gas:	Helium
Initial temp:	250ºC (on)
Pressure:	29.2 psi (on)
Total flow:	33.6 ml/min
Purge flow:	50.0 ml/min
Injection pulsed pressure:	50 psi (1.5min)
Purge flow to split vent:	30 ml/min (1.5 min)
Gas saver:	off
GC oven parameters	**Settings**
Initial temp:	0ºC
Initial hold time:	1.50 min
1st ramp:	7ºC/min
1st hold temperature:	140ºC
1st hold time:	0 min
2nd ramp:	40ºC/min:
2nd hold temperature:	220ºC
2nd hold time:	2.5 min
3rd ramp:	50ºC/min
3rd hold temperature:	240ºC
3rd hold time:	3.6 min (off)
maximum oven temperature:	260ºC
oven equilibration time:	0.01 min
Post temp:	0ºC
Post time:	0.00 min
Run time:	30.00 min
Cryo:	on
Cryo quick cooling:	on
Ambient temp:	25ºC
Cryo timeout detection:	on; 15 min
Cryo fault detection:	on
Mode:	constant flow
Outlet:	MSD
Outlet pressure:	vacuum
Column flow rate:	1.0 ml/min
Average velocity:	31 cm/sec
Transfer line temp:	225ºC (static)

Description of capillary column	Settings
Model Number:	J&W DB-VRX
Max temperature:	260ºC
Nominal length:	40.0 m
Nominal internal diameter:	0.18 mm
Nominal film thickness:	1.0 µm
Cryogenic Trap Configuration	**Settings**
Valve number:	7
Valve type:	switching
Valve status:	on
Initial time:	0 min
Initial setpoint:	7 Switched on
final time:	1.0 min
final setpoint:	7 Switched off
MS Configuration	**Settings**
MS Source:	230ºC
MS Quad:	150ºC

3. Evaluation of curve statistics

   The R-squared values for each analyte calibration curve must in all cases be greater than 0.95. In more than 90% of the cases the R-squared values are greater than 0.995. Linearity of the standard curves should be optimized through the use of universal transform (4) or the exclusion of any nonlinear portion of the curve. At least five concentration levels are used for curve fitting. Otherwise, a non-linear curve can be fit with a second order quadratic curve as long as no data points are quantified

through extrapolation. If percent deviation from the curve varies more than 20% for the lowest standard, which is weighted the most by the 1/X treatment, it should be excluded. If any of the individual calibration curves consistently have significant y-intercepts the source of this bias should be established. Possible sources include incorrect ion ratios, contamination of water/methanol used to dilute standards, contamination of analog spiking solution, and diffusion loss.

B. Usage of curve

The highest point on the calibration curve is above the expected range of results for non-occupationally exposed people and the lowest point is near or below the measurable detection limits. The other concentrations are distributed systematically between these two levels. The calibration curve spans three orders of magnitude. The values of these standards are given in Table 3.

C. Calibration verification

Calibration is performed as part of each analytical run and a calibration curve is constructed from the seven calibration standards. Additional verification is conducted by quantifying Quality Control samples of known value against the calibration curve and statistically comparing the calculated results to known values.

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Compound	PT Level 4 (ppb)	PT Level 3 (ppb)	PT Level 2 (ppb)	PT Level 1 (ppb)	Standard Deviation (ppb)	CV
1,1,1-Trichloroethane	2.560	1.280	0.640	0.320	0.027	6%
1,1,2,2-Tetrachloroethane	2.560	1.280	0.640	0.320	0.005	4%
1,1,2-Trichloroethane	2.560	1.280	0.640	0.320	0.011	5%
1,1-Dichloroethane	2.560	1.280	0.640	0.320	0.007	6%
1,1-Dichloroethene	2.560	1.280	0.640	0.320	0.019	12%
1,2-Dibromo-3-chloropropane	2.560	1.280	0.640	0.320	0.017	6%
1,2-Dichlorobenzene	2.560	1.280	0.640	0.320	0.016	7%
1,2-Dichloroethane	2.560	1.280	0.640	0.320	0.014	5%
1,2-Dichloropropane	2.560	1.280	0.640	0.320	0.014	7%
1,3-Dichlorobenzene	2.560	1.280	0.640	0.320	0.022	11%
1,4-Dichlorobenzene	2.560	1.280	0.640	0.320	0.063	6%
2,5-Dimethylfuran	2.291	1.146	0.573	0.286	0.024	7%
Benzene	2.560	1.280	0.640	0.320	0.016	5%
Bromodichloromethane	2.560	1.280	0.640	0.320	0.013	4%
Bromoform	2.560	1.280	0.640	0.320	0.022	4%
Carbon Tetrachloride	2.560	1.280	0.640	0.320	0.007	7%
Chlorobenzene	2.560	1.280	0.640	0.320	0.007	6%
Chloroform	2.560	1.280	0.640	0.320	0.013	6%
cis-1,2-Dichloroethene	2.560	1.280	0.640	0.320	0.020	9%
Dibromochloromethane	2.560	1.280	0.640	0.320	0.005	4%
Dibromomethane	2.560	1.280	0.640	0.320	0.036	7%
Ethylbenzene	2.560	1.280	0.640	0.320	0.033	7%
Hexachloroethane	2.609	1.304	0.652	0.326	0.010	6%
m-, p-Xylene	2.560	1.280	0.640	0.320	0.201	7%
Methylene Chloride	2.560	1.280	0.640	0.320	0.080	16%
Nitrobenzene	2.524	1.262	0.631	0.316	0.256	9%
o-Xylene	2.560	1.280	0.640	0.320	0.043	11%
Styrene	2.560	1.280	0.640	0.320	0.047	6%
tert-Butyl Methyl Ether	2.532	1.266	0.633	0.316	0.061	11%
Tetrachloroethene	3.123	1.562	0.781	0.390	0.124	15%
Toluene	2.560	1.280	0.640	0.320	0.064	11%
trans-1,2-Dichloroethene	2.560	1.280	0.640	0.320	0.016	7%
Trichloroethene	2.560	1.280	0.640	0.320	0.018	10%

8. Procedure Operation Instructions; Calculations; Interpretation

A. Morning startup procedure

1. Data System Startup

The data system PC may need to be reset.

a. Turn off the data system PC by using START menu and shut down.

b. After the system has restarted, log into Windows 2000 by entering the user id and password.

c. Execute the Enhanced ChemStation MS Instrument software.

2. Gas Chromatograph and Mass Spectrometer Setup

In the Instrument Control window of the ChemStation software, check the monitors for the MSD temperature and vacuum status. Monitors display the current value of a single instrument parameter and can be set to change colors if the actual parameter value varies beyond a user-determined limit from the parameter setpoint. Monitor readings should be similar to those listed in Table 10. If the vacuum, temperatures and other monitors are acceptable, the mass spectrometer can perform a background *Air and Water Check.*

Table 10. GC and MS Monitor Settings while Shutdown.M Method is Loaded.

GC Parameters	settings
Inlet temperature	250°C
Inlet pressure	42.4 psi
Inlet total flow	32.4 mL/min
Column Flow Cal.	1
Oven Temperature	100°C
Aux-2 Temperature	230
MS Parameters	**settings**
Filament	1 or 2
MS Source	230°C
MS Quad	150°C
EM Volts	0
Turbo Speed	100%

3. Air and Water Check

Run *Air and Water Check* program found in the *Diagnostics* menu and insure that relative abundance of the m/z 69 ion from the PFTBA calibration gas is at least 500,000 and that relative abundances at m/z 28 (e.g., N_2) is less than 4% and m/z 18 (e.g., H_2O) is less than 2%. If the relative abundance for m/z 69 is below 500,000 and ion optic repeller voltage profiles for PFTBA ions are acceptable as described in the MS Tuning Section 8.a.4, then increase the electron multiplier voltage. Only increase the electron multiplier voltage if the m/z 69 abundance is low for 3 days in a row as the calibration gas concentration is only approximate. If the m/z 28 and 18 are high, then identify and fix the source of the leak.

4. Mass Spectrometer Tuning

The Agilent 5973 MSD is a very stable instrument and does not require retuning unless instrument performance changes. Such changes can be seen in the PFTBA response and comparison of absolute responses of standards. However, the instrument should be retuned if the MS is brought to ambient pressure. In addition, it is critical for instrument-to-instrument and long term performance monitoring that tuning be performed only when needed.

Tuning Procedure

a. Enter preferences into *Set Tune Limits* as shown in Table 11.

Table 11. MS ion Optic Parameter Settings.

MS Ion Optic Parameter	Setting
69 Abund. target, counts	500000
Peakwidth target, amu	0.5
Maximum repeller	42.7 V
Emission current	50 V + established optimum
Maximum ion focus	127 V

b. Run *Perform MS Autotune* program and select *Autotune* method.

c. Once the autotune is complete verify that the *Repeller* ion optic voltage profiles for the PFTBA ions at m/z 69, 219 and 502 fall within the voltage limits of the ion optic power supplies. When the emission voltage is too high, the optimal responses for the PFTBA ions can exceed the *Repeller* electrode 42.7V maximum. If this case, it is necessary to lower the *Emission* voltage. If a suitable compromise cannot be established between the *Emission* and *Repeller* voltage, it is necessary to clean the ion optics.

d. Ensure that all ion optic voltages fall between optimum response for PFTBA ions at m/z 69 and 219. If voltage is out of range then adjust the voltage manually to within the proper range.

e. Once the tune has been optimized, print the ion optic profiles for the *Emission*, *Repeller*, *Ion Focus*, *Ent. Lens*, and *Ent. Lens Offset* and manually generate a tune report. These tuning data are saved in the daily run log books.

B. Analysis of samples

Blood, bovine serum QC, and water blank samples are transferred to the standard 10-mL headspace vials via 5-mL luerlock gas-tight syringes.

1. Preparation of headspace vials and syringes

a. Remove the PTFE/silicone barrier septa that have been cleaned in accordance to Section 6.c, headspace vials that have been cleaned in accordance to Section 6.b and syringes that have been cleaned in accordance to Section 6.k from their respective ovens immediately before sample preparation.

b. Assemble the headspace vial and caps by stacking a 20-mm diameter zinc-plated steel washer and PTFE/silicone barrier septum, PTFE side up, into a steel crimp top. Place the cap on the headspace vial, uncrimped.

c. Lubricate the syringe plungers with ampoulized water, assemble, and attach a 1 ½ inch 18-gauge needle.

2. Preparation of water blanks

a. Tare to 0.01 g a headspace vial with a cap on an electronic balance.

b. Withdraw 3 mL of water from a 10-mL ampoule of water prepared in accordance with Section 6.e. Lift the headspace vial cap and gently push the contents of the syringe into the vial.

c. Discharge 40 µL internal standard working solution into the water sample using the 40-µL Micro/Pettor.

d. Crimp and seal the vial. Weigh the tared headspace vial and enter the weight into the ATLIS data management system.

e. Place the headspace vial in the appropriate location in a sample tray.

f. Place used disposable glass micropipettes in a sharps container. Remove the needle from the syringe using a pair of hemostats and place it in a sharps container.

3. Preparation of bovine serum QCs

a. Using an electronic balance, tare to 0.01 g a headspace vial with a cap.

b. Withdraw 3 mL of bovine serum from a 10-mL ampoule of bovine serum prepared in accordance with Section 6.h. Lift the headspace vial cap and gently push the contents of the syringe into the vial.

c. Discharge 40 µL internal standard working solution into the bovine serum QC sample using the 40-µL Micro/Pettor.

d. Crimp and seal the vial. Weigh the tared headspace vial and enter the weight into the ATLIS data management system.

e. Place the headspace vial in the appropriate location in a sample tray.

f. Place used disposable glass micropipettes in a sharps container. Remove the needle from the syringe using a pair of hemostats and place it in a sharps container.

4. Preparation of blood samples

Blood samples are shipped on wet ice and stored at 2-6ºC until analyzed. To prepare the blood samples for analysis, they are first placed on a rotating mixer

in a level 1 (Type II Class A/B3) biological safety cabinet (BSC) at room temperature for a minimum of 15 min.

a. Using an electronic balance, tare to 0.01 g a headspace vial with a cap.

b. Lay both a blood vacutainer and syringe on the surface of the BSC and insert the needle through the stopper, lift while inverting the vacutainer/syringe couple and withdraw 3 mL of sample.

c. Remove the syringe from the vacutainer and gently dispense the blood sample into the headspace vial.

d. Discharge 40 µL of internal standard solution into the aliquoted blood sample using the 40-µL Micro/Pettor. Crimp and seal the vial. Weigh the tared headspace vial and record the weight for entry into the ATLIS data management system. The net weight is the weight of blood sample used.

e. Place the headspace vial in the appropriate location in a sample tray.

f. Place used disposable glass micropipettes in a sharps container. Remove the needle from the syringe using a pair of hemostats and place it in a sharps container.

C. Sample sequence set up

Instrument response is quantified with every sample batch. Samples are analyzed beginning with a) the standard set water blank, b) the first four lowest concentration standards in order of increasing concentration, c) an unknown-set water blank, d) a low-concentration QC, e) a high-concentration QC, f) the unknowns, g) a second unknown-set water blank, h) a second low-concentration QC, i) a high-concentration QC and finally, j) the last three standards in order of increasing concentration.

1. Logging samples into the ATLIS database management system

a. Open the ATLIS standard "GoTo" menu.

b. Log in to the application.

c. In the *Login/prep* folder execute the *Sample/Run Setup* file routine.

d. Select the *Queue Worksheet* tab at the top of the menu.

e. Select the appropriate study under the *Study* pull down menu.

f. Select the group of samples that need to be analyzed. These groups are sorted by sample age; thus, ensure that the oldest or highest priority samples are analyzed first.

g. Once the desired samples are selected, click on the *Queue BL, QC, and Standards* button. This executes a sample listing program that will allow you to select the instrument and assay by which the samples will be analyzed. Once this is selected, ATLIS automatically lists the sample names for the standards, water blanks and QC samples.

h. Select and sequence the appropriate standards, water blanks, and QC samples to create the run sequence that is to be executed. Double check the sequence list as it is difficult to change sequence order in ATLIS after it has been entered.

i. Click on the printer icon button to print and load the sample sequence sheet. This sheet includes bar codes of the sample names and run numbers. This barcode sheet is used to enter these identifiers via a barcode scanner into the PAL Cruise Control sample list table as described below in Section 8.c.2.

2. Entering sample weights into the ATLIS database management system

a. Open the ATLIS standard "GoTo" menu.

b. Log in to the application.

c. In the *Login/prep* folder execute the *Sample/Run Setup* file routine.

d. Select the *Run Log Sheet* tab at the top of the menu.

e. Click on the *Sample Prep* button.

f. Scan in the Sample ID (i.e., sample name) and Run # (i.e., input file name) from the barcode sheet.

g. Select *Single Tare Reading*.

h. Place a headspace vial with cap on the analytical balance and press the Tare button.

i. Add 3 mL of water, QC or blood sample and 40 µL ISTD.

j. Press the analytical balance enter button. This enters the tared weight of the sample into the run log file. The data system then moves on to the next sample in the sequence list.

k. When entering in the sample weight for the standards and associated water blank (i.e., BS037), scan in the weight as 3.00 gm, which is printed on the barcode sheet.

l. After the last sample is entered, verify the sample names, input the file names and the approximate weight by clicking on the *Verify Sample(s) Prepared* button.

m. Close the *Sample Prep Verification* menu.

n. Click *Update to Run Log Sheet* button.

3. Transfer Run Log Sheet Information to ATLIS and the database server

   After entering the sample weights into the run log file, transfer this information to ATLIS and the database server.

   a. Select the *Transfer Data Sequence* tab from the *Sample/Run Setup* window.

   b. Select the appropriate assay from the *Select Assay* pull down menu.

   c. Click on the Transfer Data Sequence button to transfer the run log information to ATLIS and to the database server as a *.CSV file.

4. Loading and Setup of the Agilent Enhanced ChemStation MS software

   The Enhanced ChemStation software consists of two main programs, one for instrument control (i.e., Instrument Control) and the other for sequence control (i.e., MSTop).

   a. Replace the inlet septa and inspect the inlet, inlet liner and o-ring. If the inlet contains debris such as from a broken fiber or a piece of inlet septa, then clear it away with a dry, long cotton swab. Also, replace the inlet liner and o-ring if the liner contains any debris. After this procedure has been performed wait at least 10 min before performing an *Air and Water Check*.

   b. Ensure that the SHUTDOWN.M method is loaded (refer to Appendix B for method details). This method is needed to ensure the instrument is in splitless mode and that the oven is set at 100ºC during the *Air and Water Check*.

   c. Verify instrument performance by running an *Air and Water Check* in accordance with Section 8.a.3 and attach the results into the daily sample log book.

   d. From the MSTop program load and set up the PAL Cycle Composer sequencing program.

   e. Visually inspect the SPME fiber by resetting the PAL.

   f. Creating a sample list in the Sample List menu

(1) Set up the sequence table to include the following columns; *Status, Method, Tray, Vial, FileName, CS Method, and Sample Name.*
(2) Create a sample list name using the “iyydoy" convention. This entails using the appropriate alphanumeric characters to identify the run instrument, i, the last two digits of the year, yy, and the three digit date-of-year sequence number for the day in that year, doy and the first letter of the GC/MS instrument name (e.g., B04123).
(3) Select an appropriate PAL method (i.e., Bakeout, VOC_37, or SHUTDOWN, refer to Appendix B) in the Method column.
(4) Select an appropriate tray (i.e., Tray1 or Cooler1).
(5) Input the appropriate vial tray number location in the Vial column.
(6) Input the file names.
(a)For bakeout use the bakeout”*letter*” convention for instrument and fiber conditioning (e.g., bakeouta) and bakeout”*number*” convention for conditioning between samples (e.g., bakeout1).
(b)For standard, QC and unknown samples use the iyydoy”two digit run sequence number” convention (e.g., B0412301).
(7) Select the appropriate ChemStation method in the CS Method column (i.e., Bakeout, VOC37_i, or Shutdown, refer to Appendix B).
(8) In the SampleName column, enter the sample identification information manually or by a barcode scanner as appropriate.
(a)Name the bake out runs with the same name as the file name, (e.g. bakeouta or bakeout1).
(b)Name the standard using the secondary standard label convention as described in Section 6.f.5, (e.g., SS0371).
(c)Label the blank prepared with the standard set BS037.
(d)Label the blanks prepared with the unknowns and CS BL037.
(e)Name the unknown samples with the appropriate sample identifier as determined by the study.
(f)Label the shut down run as shutdown.
(9) Place the sample tray on its appropriate tray holder.
(10) Save and print the sample list when all of the run information is entered. Place the sample list in the daily sample log.
(11) Make sure the liquid nitrogen supply valve is open.
(12) Ensure that all the vials are in the correct sample tray positions and start the run.

D. Data analysis

Samples are quantified by their analyte ion area to internal standard ion area ratio, which compensates for variable loss after sample preparation, as well as variable partitioning and SPME extraction efficiency. Peak integration, instrument response calibration and sample quantification is performed using Xcalibur Quan software (ThermoFinnigan, San Jose CA) because of enhanced data processing and archiving capabilities. Mass chromatographic data is imported from ChemStation format to Xcalibur Quan format by conversion of ChemStation format into AIA/Net CDF format. Subsequently, these CDF files are converted to Xcalibur *.RAW format using the

Xconvert feature of the Xcalibur software. This translated data is then imported into Xcalibur Quan for further examination and processing. Peak integrations are performed with the AVALON integrator and confirmed by visual inspection.

A transform is applied to linearize standard curves for those compounds where concomitant or standard responses contribute to the internal standard response. Following this, a 1/X-weighted least squares model is fit to the calibration data, where X is the concentration of the standards. Blood concentrations quantified from the standard response curves are multiplied by the appropriate dilution factor, which are determined by sample weight, to deduce measured concentrations. The detection limit is equal to three times the standard deviation at zero concentration ($3S_0$). In those cases where $3S_0$ is below the lowest standard, the lowest reportable limit is set equal to the lowest standard.

(1) Backup of Analytical Run Parameters and Results to Shared Database Server

(a) Select the *Transfer Data Sequence* tab from the *Sample/Run Setup* window.

(b) Ensure that a run folder in the appropriate instrument folder has been created through ATLIS. This folder should contain the ATLIS generated run sequence file, iiyydoy.csv

(c) Create a run folder in the appropriate instrument folder, (e.g., Archie, Bullwinkle, Rocky, etc.). For example, B04123.
i. Copy the ChemStation data folder iyydoy.s to the run folder.
ii. Copy the corresponding GC/MS and PAL parameter files to the run folder to include the atune.u, VOC37_*i*.M, *iyydoy*.psl, SPME VOC.pma, VOC37.pme.

(2) Importing ChemStation Format to Xcalibur Quan

(a) Load the ChemStation Data Analysis program on you local computer.

(b) Export data to AIA format into an AIAEXPORT.AIA folder located locally in C:/Xcalibur directory.

(c) Convert the AIA data files to *.RAW files using the XConvert1.0.exe program storing these files in the Data folder in the C:/Xcalibur directory.

(3) Creating an Xcalibur *.SLD File

(a) Open the Xcalibur *Home Page* program.

(b) Open the *Sequence Setup* program.

(c) Import the corresponding iyydoy.csv sequence file from the database server.

(d) Ensure that all columns are imported and that the *Sample Type, File Name, Sample ID, Path, Proc. Method, Level*, and *Dil Factor* are present.

(e) Ensure that all information in the *Sample Type, File Name, Sample ID, Path, Level,* and *Dil Factor* is correct. Note that the *Path* column should contain c:\xcalibur\data. The *Proc. Method* column will be empty.

(f) Load the most recent and instrument appropriate processing method in the *Proc. Method* column.

(g) Save the *.SLD file using the *iyydoy*.sld naming convention.

(h) Perform the initial data processing by running the Batch Process routine. This routine will generate *.RST files corresponding to each *.RAW file in the C:/Xcalibur/data folder.

(4) Updating and creating an Xcalibur *.PMD file

(a) From the Xcalibur Home Page menu open the Processing Setup program.

(b) From the main menu select the *Identification* tab.
   i. Enter the ATLIS designated compound name as given in Table 12.
   ii. In the pull-down menus select *MS* as the detector type, *Avalon* as the peak detect, and *Mass Range* as the trace.
   iii. Enter the corresponding *Mass Range (m/z)* and *Retention Time, Expected (min)*. The *Window* and *View width* may be left at the default values of 10 sec and 1.0 min., respectively.

(c) From the main menu select the *Detection* tab.
   i. Set the *Start, End and Area Threshold* values low at 5 and increase, as needed, to prevent over-integration of area beyond the peak.
   ii. Avoid using smooth, but instead use *Bunching Factor* to extend the integration range.
   iii. *Tension* may be increased above 0 to pull the integrator baseline down or more parallel with the x axis. This function is helpful when the integrator is uncertain when the peak starts or ends because of fronting or tailing.

(d) From the main menu select the *Calibration* tab.
   i. Select the appropriate *Component type*.
   ii. Configure the curve fitting parameters as follows: *weighting* as 1/x, *origin* as ignore and *response* as area.
   iii. For the primary and quantification ions select the appropriate internal standard compound.

iv. If known the target compound-to-ISTD factor may be entered in the *Isotope %* dialog box.

(e) From the main menu select the *Levels* tab.
   i. Enter the appropriate concentrations for the standards as listed in Table 3. Designate standard SS0371 as level 1, SS0372 as level 2, etc.
   ii. Enter the appropriate concentrations for the QC samples as listed in Table 8. Designate the low concentration QC sample as level 1 and the high concentration QC sample as level 2.

(f) When all the needed information has been entered save the file with the immddyy convention (e.g., B041204).
   i. Enter the appropriate concentrations for the standards as listed in Table 3. Designate standard SS0371 as level 1, SS0372 as level 2, etc.

(5) Creating an Xcalibur *.XQN file

(a) From the Xcalibur Home Page menu open the Quan Browser program.

(b) Open the *.SLD file in the C:/Xcalibur/data folder.

(c) Save the opened *.SLD file as a iyydoy.xqn file.

Table 12. Xcalibur analyte names and examples of corresponding SIM ions, retention times and target compound to ISTD correction factors used in creating the *.PMD.

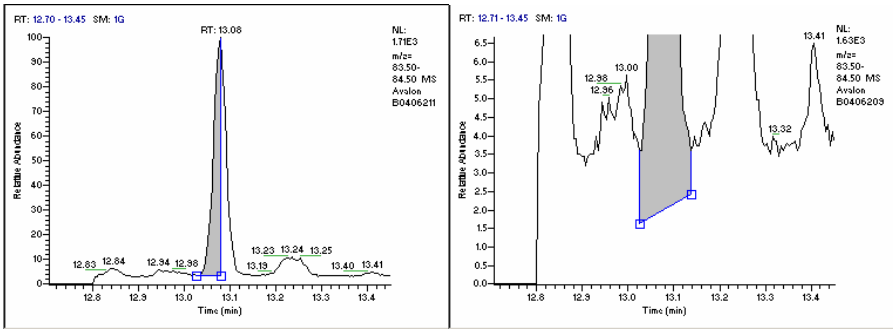
ATLIS code	Xcalibur *.PMD Analyte Name	ISTD Ion	rt (min)	Quant Ion	Conf Ion	rt (min)	TC to ISTD correction (%) Quant.	Conf.
VFN	FURAN	72	7.68	68	39	7.71	0	0
V1E	1_1_DICHLOROETHYLENE	100	8.24	96	98	8.24	10.1	15.6
VAC	ACRYLONITRILE	56	8.44	53	52	8.44	0	0
VMC	METHYLENE_CHLORIDE	85	8.55	84	49	8.55	3.3	3
V2T	TRANS_1_2_DICHLOROETHYLENE	100	9.62	96	98	9.61	10.1	15.9
VME	T_BUTYL_METHYL_ETHER	82	9.73	73	57	9.96	9.8	0
V1A	1_1_DICHLOROETHANE	66	9.98	63	65	10.06	0	0
V06	HEXANE	58	10.74	86	41	10.74	23.7	5.4
V2C	CIS_1_2_DICHLOROETHYLENE	100	10.96	96	98	10.96	9.9	15.6
VCF	CHLOROFORM	86	11.32	83	85	11.32	1.2	1.8
V2A	1_2_DICHLOROETHANE	67	12.18	62	64	12.30	0	0
VTE	1_1_1_TRICHLOROETHANE	102	12.34	97	99	12.43	0	0
VCT	CARBON_TETRACHLORIDE	120	12.92	117	119	12.99	1	1
VBZ	BENZENE	84	13.08	78	77	13.08	0	0
VDM	DIBROMOMETHANE	178	13.83	174	93	13.97	0	0
VDP	1_2_DICHLOROPROPANE	67	13.95	63	76	14.04	0	0
VTC	TRICHLOROETHYLENE	133	14.03	130	132	14.03	2.1	2.1
VBM	BROMODICHLOROMETHANE	86	14.20	83	85	14.20	0.9	1.4
2DF	2_5_DIMETHYLFURAN	98	14.49	96	95	14.49	0.4	0.5
V2E	1_1_2_TRICHLORETHANE	102	16.16	97	83	16.24	0	0
VTO	TOLUENE	98	16.56	91	92	16.56	0	0
VCM	DIBROMOCHLOROMETHANE	130	17.00	129	127	17.00	1.5	2
VDE	DIBROMOETHANE	111	17.41	107	109	17.41	0	0
V4C	TETRACHLOROETHYLENE	169	17.78	166	164	17.78	1	1.3
VCB	CHLOROBENZENE	118	18.95	112	77	18.95	0	0
VEB	ETHYLBENZENE	97	19.37	91	106	19.37	0	0.1
VXY	M_/P_XYLENE	97	19.73	91	106	19.73	0	0.1
VBF	BROMOFORM	174	19.81	173	175	19.81	2.1	4.1
VST	STYRENE	110	20.31	104	103	20.31	0	0
V4E	1_1_2_2_TETRACHLOROETHANE	86	20.42	83	85	20.37	0	0
VOX	O_XYLENE	112	20.46	91	106	20.34	0	0
V3B	1_3_DICHLOROBENZENE	152	22.97	111	150	22.97	0	0
VDB	1_4_DICHLOROBENZENE	152	23.05	111	150	23.05	0	0
V1D	1_2_DICHLOROBENZENE	152	23.39	111	150	23.39	0	0
V2P	1_2 _DIBROMO_ 3_ CHLOROPROPANE	158	23.83	157	155	23.83	4.2	5
VHE	HEXACHLOROETHANE	204	23.89	201	166	23.89	1.3	3.3
VNB	NITROBENZENE	129	24.04	123	77	24.04	0	0
VHB	HEXACHLOROBUTADIENE	229	25.46	225	223	25.46	20.4	23.6

E. Verifying and Correcting of Xcalibur Data Processing

1. Visually inspect each peak and correct peak integrations that have been performed erroneously by the AVALON integrator as shown in Figure 1. Ensure that the

integration approach for standards, blanks, QCs and unknowns is consistent for a specific target ion.

Figure 1. Examples of peak integrator algorithm failure.



2. If the absolute ion signal to noise is not at least a factor of 2, set the peak status to not found or if the response is less than SS0371 then leave as *Response Low*.

3. Ensure that the *primary quantification* ion responses are similar to those of the confirmation ion. Reasons for a difference would be error in error in integration of the primary quantification or confirmation ion, an error in curve fitting of standards, or a wrong *Target Compound to ISTD* correction factor, etc.

4. Evaluate the exclusion list and verify that the *percent difference* between the *calculated* amount and *expected* amount for all standards is below 5%. If a standard is above 5%, verify proper integration of that standard.

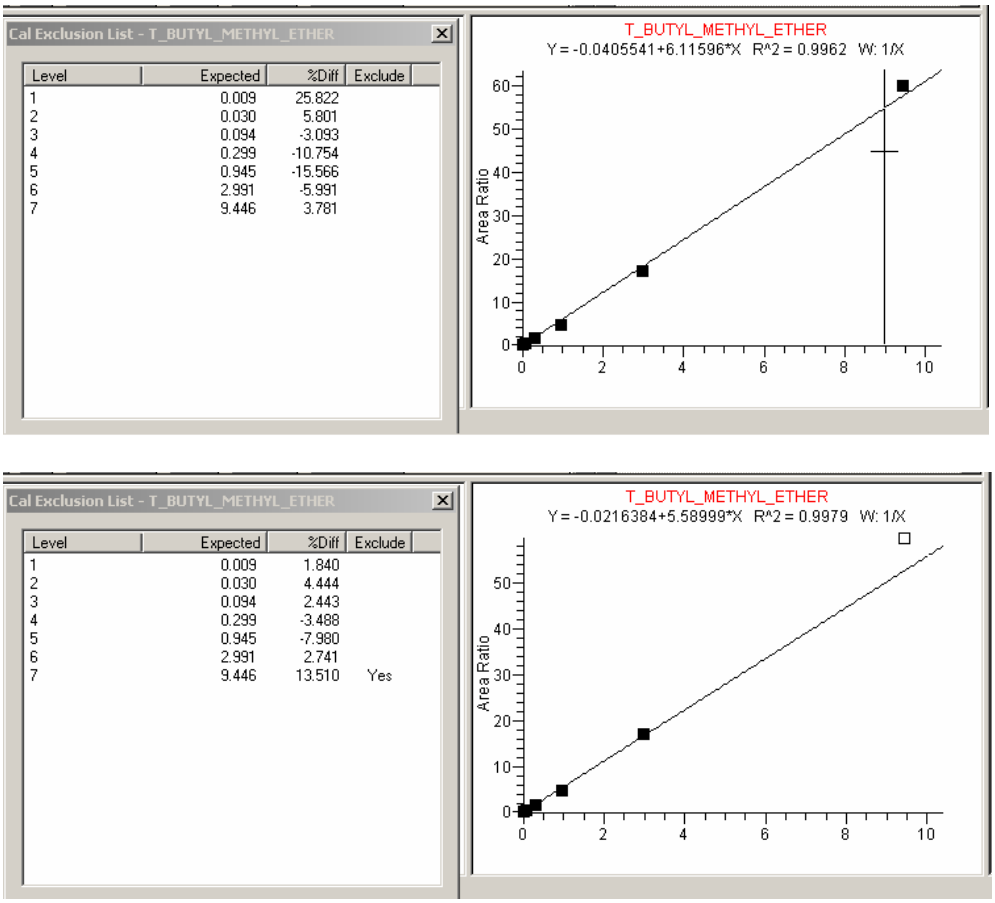
5. Excluding standards data

   a. If the percent difference is greater than 20%, determine if exclusion would change the unknown results by more than 5%. If not, keep the data point. If exclusion significantly affects the results, then identify the cause that would affect only that standard before excluding that data point. Scenarios that might only affect a single standard include a poor seal on a headspace vial, a cracked secondary standard ampoule, no addition of internal standard, contamination of the standard set during storage, *etc*.

   b. Exclude standards SS0376 and/or SS0377 because of curve “curl-up effect” if all unknowns are below standard SS0375 in concentration. If standards SS0376 or SS0377 are needed to quantify high concentration unknowns, then select the better fit that is achieved between a linear and a second-order polynomial.

      The “curl up” effect occurs when the highest concentration standards positively deviate from a linear fit established by the lowest concentration standards. Not excluding these data will result in significant positive deviation of the lower standards as demonstrated in Figure 2.

Figure 2. Example of "Curl up" effect as found with methyl-*tert*-butyl ether



6. Compare the uncorrected ISTD absolute response and ensure consistency among the standards, blanks, QCs and unknowns. An unusually high internal standard level might occur if the ISTD is added twice. A low or absent ISTD response might be indicative of no ISTD addition, a cracked vial, a poor cap seal, *etc*. If the ISTD response is out of range then exclude the sample and rerun it if it is an unknown.

7. *Optimizing Target Compound to ISTD correction*

   a. Select the value that minimizes the standard SS0371 percent difference as defined above in Section 8.e.4.

   b. If the response from SS0372 is greater than 5 percent then divide the error evenly between SS0371 and SS0372.

   c. If increments made to the *Target Compound to ISTD* correction create too large of a change to SS0371 percent difference, then SS0377 may be excluded if not needed.

F. Transferring data to the ATLIS database

1. From the Xcalibur Quan Browser Program load the corrected *.XQN file in accordance with Section 8.e and export the data as an *Excel long report*.

2. Save the Excel long report using the iyydoy.xls file naming convention.

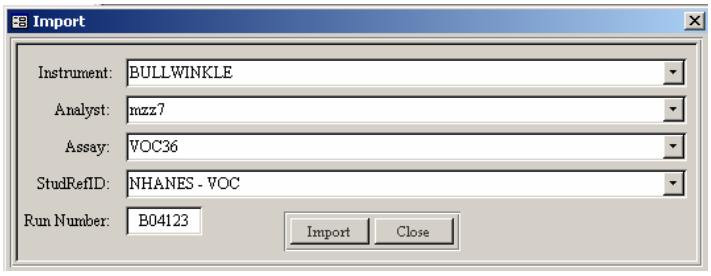
3. Open ATLIS using the standard "GoTo" menu.

4. Login.

5. In the *Login/prep* folder execute the *Import Results File* routine.

6. As shown in Figure 3, import the run information in the import dialog box and select the appropriate item in the pull down menu for the instrument, the import analyst, assay, and the study reference identifier. Enter the run identifier using the *iyydoy* convention.

Figure 3. Example of settings to be entered into the import dialog box.



7. Browse to or enter the name of the Xcalibur Long Excel Report (XLER) .XLS file that corresponds to the *.XQN file.

8. Once the *Import Table* is loaded, change all the analysis dates in the *AnalysisDate* column to match the "Julian" date in the run identifier using the Find and Replace tool. For example the date for B04123 is 5/2/2004.

9. Click on the *Find X's* button. This routine compares run information on each sample with that entered during sample log in. If any Xs are found ensure that the proper information appears in each column for that sample. The study reference identifier may need to be changed if samples from different studies occur in the same run. Otherwise contact the QC officer to resolve other conflicts.

10. When the import is ready to transfer, click on the *Transfer* button.

G. Formal Quality Control Material Evaluation

Quality control sample results are formally evaluated by an independent quality control officer following import of data into the ATLIS relational database. The QC samples

analyzed with a batch of data are evaluated against characterization means and standard deviation limits determined by the QC officer. The QC samples are evaluated using modified Westgard rules as specified by DLS SAS program. Any failure of QC rules for an analyte results in rejection of the corresponding data for that analyte on the specific day in question. Once the source of the QC problem is identified, the samples are subsequently reanalyzed.

H. Additional Quality Assurance Data Evaluation

Other quality parameters are examined in addition to evaluation of quality control specimen for acceptable precision and accuracy. These include evaluation of confirmation ion ratios to confirm that detectable results in unknown samples also contain an appropriate level of a confirmatory ion signal. Confirmation ion ratios for unknowns can not deviate in excess of 20% from the confirmation ion ratios determined from reference analyte material. Adequate internal standard response is used to confirm adequate sensitivity for the instrumentation. Contamination is evaluated by comparing calculated amounts in method blanks to detection limits.

I. Unknown samples repeat limits

Unknown blood samples that yield concentrations higher than the 95 percentile of the accumulated National Health and Nutrition Examination Survey (NHANES) population are repeated. This action is performed to ensure that the sample was not inadvertently contaminated throughout the sample preparation process. Repeat limits are listed in Table 13 along with pertinent NHANES statistical data.

Table 13. Repeat limits for Unknown Blood Samples

Compound	Median	Conc. at 90 percentile rank	Conc. at 95 percentile rank	Conc. at 99 percentile rank	Repeat Limit (ng/mL)
Furan	NA	NA	NA	NA	NA
1,1-Dichloroethylene	<0.009	<0.009	<0.009	<0.009	0.009
Acrylonitrile	NA	NA	NA	NA	NA
Methylene Chloride	<0.07	<0.07	0.100	0.130	0.130
trans-1,2-Dichloroethylene	<0.009	<0.0092	<0.0092	<0.0092	0.009
tert-Methyl-Butyl Ether	0.020	0.126	0.182	0.339	0.339
1,1-Dichloroethane	<0.010	<0.010	<0.010	<0.010	0.010
n-Hexane	NA	NA	NA	NA	NA
cis-1,2-Dichloroethylene	<0.010	<0.010	<0.010	<0.010	0.010
Chloroform	0.013	0.050	0.070	0.158	0.158
1,2-Dichloroethane	<0.009	<0.009	<0.009	0.011	0.011
1,1,1-Trichloroethane	<0.048	<0.048	<0.048	0.068	0.068
Carbon Tetrachloride	<0.005	0.028	0.033	0.086	0.086
Benzene	0.051	0.240	0.404	0.737	0.737
Dibromomethane	<0.030	<0.030	<0.030	0.100	0.100
1,2-Dichloropropane	<0.008	<0.008	<0.008	<0.008	0.008
Trichloroethylene	<0.012	<0.0124	0.013	0.033	0.033
Bromodichloromethane	<0.006	0.008	0.012	0.026	0.026
2,5-dimethylfuran	<0.012	0.087	0.130	0.280	0.280
1,1,2-Trichloroethane	<0.010	0.010	0.010	0.010	0.010
Toluene	0.150	0.614	0.980	2.050	2.050
Dibromochloromethane	0.001	0.005	0.009	0.016	0.016
1,2-Dibromoethane	NA	NA	NA	NA	NA
Tetrachloroethylene	0.048	0.110	0.240	1.300	1.300
Chlorobenzene	<0.011	<0.011	<0.011	<0.011	0.014
Ethylbenzene	0.033	0.097	0.133	0.240	0.240
m/*p*-Xylene	0.150	0.476	0.622	1.100	1.100
Bromoform	0.002	0.006	0.021	0.086	0.086
Styrene	0.040	0.130	0.180	0.352	0.352
1,1,2,2-Tetrachloroethane	<0.010	<0.010	<0.010	<0.010	0.010
o-Xylene	0.049	0.097	0.122	0.273	0.122
1,3-Dichlorobenzene	<0.050	<0.050	<0.050	<0.050	0.050
1,4-Dichlorobenzene	0.142	2.215	6.600	31.200	31.200
1,2-Dichlorobenzene	<0.100	<0.100	<0.100	<0.100	0.100
1,2-Dibromo-3-chloropropane	<0.100	<0.100	<0.100	<0.100	0.100
Hexachloroethane	<0.011	<0.011	<0.011	<0.011	0.011
Nitrobenzene	<0.300	1.000	1.000	1.000	1.000
Hexachlorobutadiene	NA	NA	NA	NA	NA

9. Reportable Range of Results

A. Linearity Limits

The following table gives the reportable range of results for the analytes detectable using this method. The lower reportable limit is either the detection limit or the lowest standard whichever is lower. The upper reportable limit is the highest linear standard.

Analyte Name	Lowest Reportable Limit (ppb)	Highest Reportable Limit (ppb)
1,1,1-Trichloroethane	0.048	22.7789
1,1,2,2-Tetrachloroethane	0.0099	4.823
1,1,2-Trichloroethane	0.007	7.4189
1,1-Dichloroethane	0.005	4.7155
1,1-Dichloroethylene	0.0094	8.064
1,2-Dichlorobenzene	0.07	7.3574
1,2-Dichloroethane	0.009	9.2928
1,2-Dichloropropane	0.0076	7.3114
1,3-Dichlorobenzene	0.03	6.4512
1,4-Dichlorobenzene	0.12	38.2464
2,5-dimethylfuran	0.0112	11.2589
Benzene	0.0242	11.8579
Bromodichloromethane	0.0136	12.0269
Bromoform	0.0196	19.9834
Carbon Tetrachloride	0.0049	4.8845
Chlorobenzene	0.0114	4.7462
Chloroform	0.0109	9.001
cis-1,2-Dichloroethylene	0.0094	9.2621
Dibromochloromethane	0.0046	4.608
Dibromomethane	0.03	18.3245
Ethylbenzene	0.0243	12.1344
Hexachloroethane	0.011	5.8214
m-/p-Xylene	0.0335	16.1126
Methylene Chloride	0.07	17.4797
o-Xylene	0.0494	6.0979
Styrene	0.03	26.2656
Methyl-tert-Butyl Ether	0.04	20.0448
Tetrachloroethylene	0.0475	21.9494
Toluene	0.0246	12.288
trans-1,2-Dichloroethylene	0.0092	9.1238
Trichloroethylene	0.0124	5.6064

B. Analytical Sensitivity

Detection limits for these analytes are given in the following table. These values were determined by calculating the standard deviation at each standard concentration following repeated measurements of the standards. These standard deviations were then plotted versus concentration (4). The y-intercept of the least squares fit of this line equals S_0, with 3 S_0 being the calculated detection limit (4). The detection limits are generally in the low ppt range.

C. Accuracy

Since volatile organic compounds are not stable for extended periods in blood, no standard reference material is available. The accuracy basis for this method is established by determining the recovery of spiked blood samples. In order to examine the consistency of this recovery over the range of levels encountered in blood, these measurements were taken at different concentrations. The results of these

measurements are given in the following tables. The recoveries at most individual spiking levels fall between 75 and 150%. These results are consistent over the entire range of added analyte, including many measurements which were performed close to the detection limits.

D. Precision

The results of repeated measurements on spiked blood samples are also given in the following tables. Relative standard deviations are in most cases less than 30%. As expected, most of the exceptions were found in the low spike samples. These standard deviation results are actually higher than would be encountered in typical blood determinations since they include variation in the blood both before and after spiking. Multiple measurements on spiked QC materials show somewhat lower standard deviation results, averaging 19.4% for all analytes combined.

E. Analytical Specificity

Analytical specificity is established by comparing the ratios of the areas of analyte ion chromatographic peaks with those of confirmation ions along with reproducible GC retention times. The combination of these two measures ensures excellent analytic specificity.

Additional steps are also critical in promoting analytical specificity by removing extraneous compounds from the sample analysis system. Interferences which have their source in the measurement apparatus itself are examined by measuring instrument blanks. Blank samples are measured at least twice every day for this purpose. Glassware used for standards is treated to remove possible interferences and contamination.

The water used for dilution of standards and as water blanks is an extremely critical potential source of interference. No commercial filtering or purification system was found which could consistently yield water with acceptably low levels of VOCs (< 20 ppt for most analytes). An acceptable commercial source of water has been identified, but this must be screened for acceptable lots. Under some circumstances even this source of water failed to yield acceptable levels of volatile organic compounds. In this case, the water is further purified by helium refluxing to yield blank water with acceptable levels of VOCs. To prevent further contamination from the laboratory air, water samples are sealed in glass ampules. In all cases, typical blank water levels are below the detection limits given above.

Recovery and Reproducibility Results for VOCs in Whole Blood

Compound	Detection Limit (ppb)	Mean (ppb)	Number	Standard Deviation (%)	Recovery (%)
1,1,1-Trichloroethane	0.0245	0.29	4	5.8	121
		2.4	4	15	132
		12	4	29	105
1,4-Dichlorobenzene	0.0381	0.46	4	7.5	96
		3.7	4	5.1	100
		19	4	9.2	92
2,5-Dimethylfuran	0.0171	0.12	4	3.6	73
		0.94	4	9.5	67
		4.9	4	18	55
Benzene	0.0222	0.15	4	4.3	121
		1.2	4	9.9	118
		6.2	4	17	96
Carbon Tetrachloride	0.0045	0.053	4	4.5	132
		0.43	4	15	152
		2.3	4	31	111
Chloroform	0.0339	0.11	3	11	111
		0.87	3	16	106
		4.6	3	7.2	75
Ethylbenzene	0.012	0.14	4	4.6	90
		1.2	4	7.6	91
		6.1	4	11	87
Methylene chloride	1.25	0.23	4	22	116
		1.8	4	6	100
		9.7	4	18	84
Styrene	0.008	0.096	4	4.9	97
		0.78	4	7.2	106
		4.1	4	11	98
tert-Butyl Methyl Ether	0.023	0.27	4	4.9	111
		1.1	4	5.8	120
		2.2	4	7.7	125
Tetrachloroethene	0.0196	0.23	4	7	139
		1.9	4	11	157
		10	4	15	124
Toluene	0.0684	0.11	4	14	104
		0.91	4	5.8	119
		4.8	4	13	99
Trichloroethene	0.0063	0.049	4	5.6	110
		0.4	4	14	122
		2.1	4	17	97
m-/p-Xylene	0.0261	0.22	4	7.4	118
		1.8	4	7.4	134
		9.5	4	11	118
o-Xylene	0.0246	0.095	4	4.9	97
		0.78	4	7.2	106
		4.1	4	11	98

10. Quality Control (QC) Procedures

A. Quality Assessment

Quality assurance and quality control procedures follow standard practices (4). Daily experimental checks are made on the stability of the analytical system and standards and quality control materials, which are added to each day's run sequence. At least three quality assessment samples are analyzed in each run that include a water blank prepared with the unknown blood samples (i.e., BL037) and two QC samples at different concentrations. In addition to these samples, other QC samples may be added to evaluate assay performance that include a water blank prepared with the standards and additional blank water and QC samples prepared with the unknown blood samples. Absolute labeled-internal standard response and their retention times from the first water blank are compared with that from previous runs to check method and instrument performance. All data entry errors are evaluated by the supervisor and corrected only after consultation with the analyst and positive identification of the correct information.

B. Quality Control Procedures

1. Establishing QC limits

Quality control limits are established by characterizing assay precision with 20 distinct analyses of each QC pool. Two different pools of quality control material are used, QC low and QC High. Different variables are included in the analysis (e.g., different sets of Standards and Internal standards and 20 different sets of QC low and high) to capture realistic assay variation over time. The mean, standard deviations (i.e., within run, among run, and overall), and control limits are determined from this QC characterization data set. Individual quality control charts for the characterization runs are created, examined, and quality control limits are used to verify assay precision and accuracy on a daily basis.

C. Proficiency Testing

1. Scope of PT

The proficiency testing (PT) scheme for this method is administered by an in-house Proficiency Testing Coordinator. The samples are analyzed and the results evaluated by the in-house PT coordinator.

2. Frequency of PT

Five samples of unknown PT concentrations are analyzed twice a year using the same method described for unknown samples.

3. Documentation of PT

Analytical PT results are reviewed by the analyst and laboratory supervisor, and then submitted to the in-house PT Coordinator electronically. The PT results are evaluated by the PT Coordinator; the analysis passes proficiency testing if ≥ 80% of the results deviate ≤ 25% from the known value. A summary report of the PT evaluation is maintained by the laboratory supervisor. If the assay fails proficiency testing then the sample preparation and instrumentation are thoroughly examined to identify and correct the source of assay error. Unknown specimens are not analyzed until the method successfully passes proficiency testing.

11. Limitations of Method; Interfering Substances and Conditions

This method is an isotope dilution mass spectrometry method, widely regarded as the definitive method for the measurement of organic toxicants in human body fluids. Alteration of particular aspects of this method can result in major interferences. Care is required in order to produce non-contaminated blanks, vacutainers, and quality control materials. The range of linearity and limits of detection are given above in Sections 9.a. and 9.b., respectively.

12. Reference Ranges (Normal Values)

Reference ranges for VOCs have been measured in a sample of 700 to 1000 persons selected from the Third National Health and Nutrition Examination Survey (NHANES III). The sample is not representative of the U.S. population but it is designed to examine the influence of age, sex, race/ethnicity, urban/rural status and region of the country on VOC levels.

Detectable analyte levels of 11 VOCs have been found in 75% or more of the samples from this reference population. For these 11 VOCs, statistical results are given below.

Blood levels of volatile organic compounds in a reference range of the non-occupationally exposed U.S. population

Analyte	Detection Limit (ppb)	Number	Mean (ppb)	Median (ppb)	Percentiles 5th (ppb)	Percentiles 95th (ppb)
1,1,1-Trichloroethane	0.086	574	0.34	0.13	ND*	0.8
1,4-Dichlorobenzene	0.073	1037	1.9	0.33	ND	9.2
2-Butanone	0.5	1101	7.1	5.4	1.9	16.9
Acetone	200	1062	3100	1800	640	>6000
Benzene	0.03	589	0.13	0.061	ND	0.48
Ethylbenzene	0.02	631	0.11	0.06	ND	0.25
Styrene	0.019	657	0.074	0.041	ND	0.18
Tetrachloroethene	0.03	590	0.19	0.063	ND	0.62
Toluene	0.092	604	0.52	0.28	0.11	1.5
m-/p-Xylene	0.033	649	0.37	0.19	0.074	0.78
o-Xylene	0.04	711	0.14	0.11	0.044	0.3

***** Result below detection limit

The following table gives the percent of blood samples found above the detection limits for those analytes which were detectable in 10 to 75% of the samples we analyzed from NHANES III.

Percent of samples with blood levels above detection limit for volatile organic compounds in a reference range of the non-occupationally exposed U.S. population.

Analyte	Detection Limit (ppb)	Number	Percent of Population Above Detection Limit
Bromodichloromethane	0.009	1072	14
Chlorobenzene	0.007	1024	21
Chloroform	0.021	979	54
Chlorodibromomethane	0.013	1035	12
Trichloroethene	0.01	677	13

A number of other analytes were also examined in this reference range study but were found at detectable levels in fewer than 10% of the samples examined. These analytes along with their detection limits were 1,1,2,2-tetrachloroethane, 0.008 ppb; 1,1,2-trichloroethane, 0.016 ppb; 1,1-dichloroethane, 0.009 ppb; 1,1-dichloroethene, 0.018 ppb; 1,2-dichlorobenzene, 0.044 ppb; 1,2-dichloroethane, 0.012 ppb; 1,2-dichloropropane, 0.008 ppb; 1,3-dichlorobenzene, 0.019 ppb; bromoform, 0.027 ppb; carbon tetrachloride, 0.019 ppb; cis-1,2-dichloroethene, 0.013 ppb; dibromomethane, 0.044 ppb; hexachloroethane, 0.079 ppb; methylene chloride, 0.089 ppb; and trans-1,2-dichloroethene, 0.014 ppb.

13. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

A. Internal reference area counts

If the labeled ion counts of the blank samples fall below 50% of the median of these values, this indicates that the instrumental sensitivity has fallen below tolerable limits. The following steps should be taken and the instrument sensitivity rechecked after each is performed. Once sensitivity has been reestablished further steps are not necessary.

1. Perform and Air and Water Check as described in Section 8.a.3.

2. Evaluate the instrument tuning parameters as described in Section 8.a.4.

3. Remove and clean the mass spectrometer source. Replace the filament and any ceramics which may be conducting.

4. Test the electron multiplier gain and replace this component if it has markedly decreased.

B. Analyte in blank material

If an inordinately large amount of analyte is measured in the blank, but this is not seen in the remainder of the samples, this indicates a temporary contamination of the blank. The source of this incident should be investigated to prevent repeat occurrences but, no further action is required.

C. Analyte in all samples

If an inordinately large amount of analyte is present in all measurements for a particular day, either the labeled analog solution is contaminated or there is a continual source of contamination. The following steps should be taken until the contamination is removed.

1. Check the immediate area of the mass spectrometer and the laboratory where standards are made for use of the contaminating agent.

2. Discard the purge & trap grade methanol used for dilution of the internal standard. For further analyses use a new bottle of purge & trap grade methanol.

3. Replace all syringe clean-up materials.

D. QC sample outside of 99% confidence limits

If one or more of the quality control sample concentration results fall outside the 99% limits, one of the above is the most likely cause. Follow the steps outlined above to isolate and correct the problem. Note that in all cases the supervisor should be consulted for the appropriate corrective actions. No analytical results may be reported for runs not in statistical control.

14. Limitations of Method; Interfering Substances and Conditions

This method is an isotope dilution mass spectrometry method, widely regarded as the definitive method for the measurement of organic toxicants in human body fluids. Alteration of particular aspects of this method can result in major interferences. Care is required to produce non-contaminated blanks, vacutainers, and quality control materials. The quantification range and limits of detection are given above in Section 9.

15. Reference Ranges (Normal Values)

Reference ranges for VOCs have been measured in a sample of 700 to 1000 persons selected from the Third National Health and Nutrition Examination Survey (NHANES III). The sample is not representative of the U.S. population but it is designed to examine the influence of age, sex, race/ethnicity, urban/rural status and region of the country on VOC levels.

Detectable analyte levels of 11 VOCs have been found in 75% or more of the samples from this reference population. For these 11 VOCs, statistical results are given in Table 16.

Table 16. Blood levels of volatile organic compounds in a reference range of the non-occupationally exposed U.S. population.

Analyte	Detection Limit (ppb)	Number	Mean (ppb)	Median (ppb)	Percentiles 5th (ppb)	95th (ppb)
1,1,1-Trichloroethane	0.086	574	0.34	0.13	ND*	0.8
1,4-Dichlorobenzene	0.073	1037	1.9	0.33	ND	9.2
2-Butanone	0.5	1101	7.1	5.4	1.9	16.9
Acetone	200	1062	3100	1800	640	>6000
Benzene	0.03	589	0.13	0.061	ND	0.48
Ethylbenzene	0.02	631	0.11	0.06	ND	0.25
Styrene	0.019	657	0.074	0.041	ND	0.18
Tetrachloroethene	0.03	590	0.19	0.063	ND	0.62
Toluene	0.092	604	0.52	0.28	0.11	1.5
m-/p-Xylene	0.033	649	0.37	0.19	0.074	0.78
o-Xylene	0.04	711	0.14	0.11	0.044	0.3

* Result below detection limit

Table 17 gives the percent of blood samples found above the detection limits for those analytes, which were detectable in 10 to 75% of the samples we analyzed from NHANES III. A number of other analytes were also examined in this reference range study but were found at detectable levels in fewer than 10% of the samples examined. These analytes along with their detection limits were 1,1,2,2-tetrachloroethane, 0.008 ppb; 1,1,2-trichloroethane, 0.016 ppb; 1,1-dichloroethane, 0.009 ppb; 1,1-dichloroethene, 0.018 ppb; 1,2-dichlorobenzene, 0.044 ppb; 1,2-dichloroethane, 0.012 ppb; 1,2-dichloropropane, 0.008 ppb; 1,3-dichlorobenzene, 0.019 ppb; bromoform, 0.027 ppb; carbon tetrachloride, 0.019 ppb; cis-1,2-dichloroethene, 0.013 ppb; dibromomethane, 0.044 ppb; hexachloroethane, 0.079 ppb; methylene chloride, 0.089 ppb; and trans-1,2-dichloroethene, 0.014 ppb.

Table 17. Percent of samples with blood levels above detection limit for volatile organic compounds in a reference range of the non-occupationally exposed U.S. population.

Analyte	Detection Limit (ppb)	Number	Percent of Population Above Detection Limit
Bromodichloromethane	0.009	1072	14
Chlorobenzene	0.007	1024	21
Chloroform	0.021	979	54
Chlorodibromomethane	0.013	1035	12
Trichloroethene	0.01	677	13

16. Critical-Call Results (“Panic” Values)

The health effects resulting from exposure to low levels of VOCs is currently unclear. The method described here is designed for the measurement of low level exposure to VOCs, thus panic values will not be measured with this method.

17. Specimen Storage and Handling During Testing

Specimens may reach and maintain ambient temperature during analysis. If the measurement is delayed to the next day, samples can be left on a cooled sample tray at 15 $\pm$ 1^{o}C. Samples are not placed in a refrigerator that has not been recently vented. Most sample queues run for extended time periods of up to 24-hr duration. As a precaution biological samples (unknowns and QC) are racked into a chilled tray (15 $\pm$ 1^{o}C) while awaiting analysis.

18. Alternate Methods for Performing Test and Storing Specimens if Test System Fails

The analysis of VOCs in whole blood at parts-per-trillion levels is an extremely complex measurement. There are no acceptable alternative methods for this analysis. If the analytical system fails, storage of unprepared and partially prepared specimens at 2-6°C is recommended up to 24 hr.

A. Length of Time Samples may be banked

Repeat measurements of samples stored at 2-6^{o}C indicate that whole blood VOC samples may be banked for at least 7 weeks. Because these are whole blood samples, longer storage results in samples which are harder to manipulate and produce additional analytical problems. Thus, even though analytical results may not change over this time, samples may be less amenable to analysis. Volatile organic compounds occur naturally in the body, and metabolism may alter their concentration with storage.

B. Proper banking procedures

Whole blood samples for VOC measurement should be stored in the dark at 2–6^{o}C. This prevents blood cell rupture which would occur during freezing. In addition, freezing of blood can lead to breakage of vacutainers and loss of sample in some cases. Since VOCs are lost whenever the containers in which they are stored are opened, it is not appropriate to transfer the blood samples to another container which would be more resistant to breaking.

17. Test-Result Reporting System; Protocol for Reporting Critical Calls (if Applicable)

Results are generally reported to 2 significant digits. In addition, reports of reference range means and medians should also accompany all reports because these values are not available elsewhere.

The health effects resulting from exposure to low levels of VOCs is currently unclear. Therefore no critical call levels are set.
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18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

If greater than 3 mL of sample remain after analysis, this material should be returned to storage at 2-6^{o}C.

Standard record keeping means (database, sample logs, optical disc files) are used to track specimens. It is recommended that records be maintained for 3 years, including related QA/QC data, and that duplicate records be kept off-site in electronic format. All personal identifiers should be available only to the medical supervisor to maintain confidentiality.

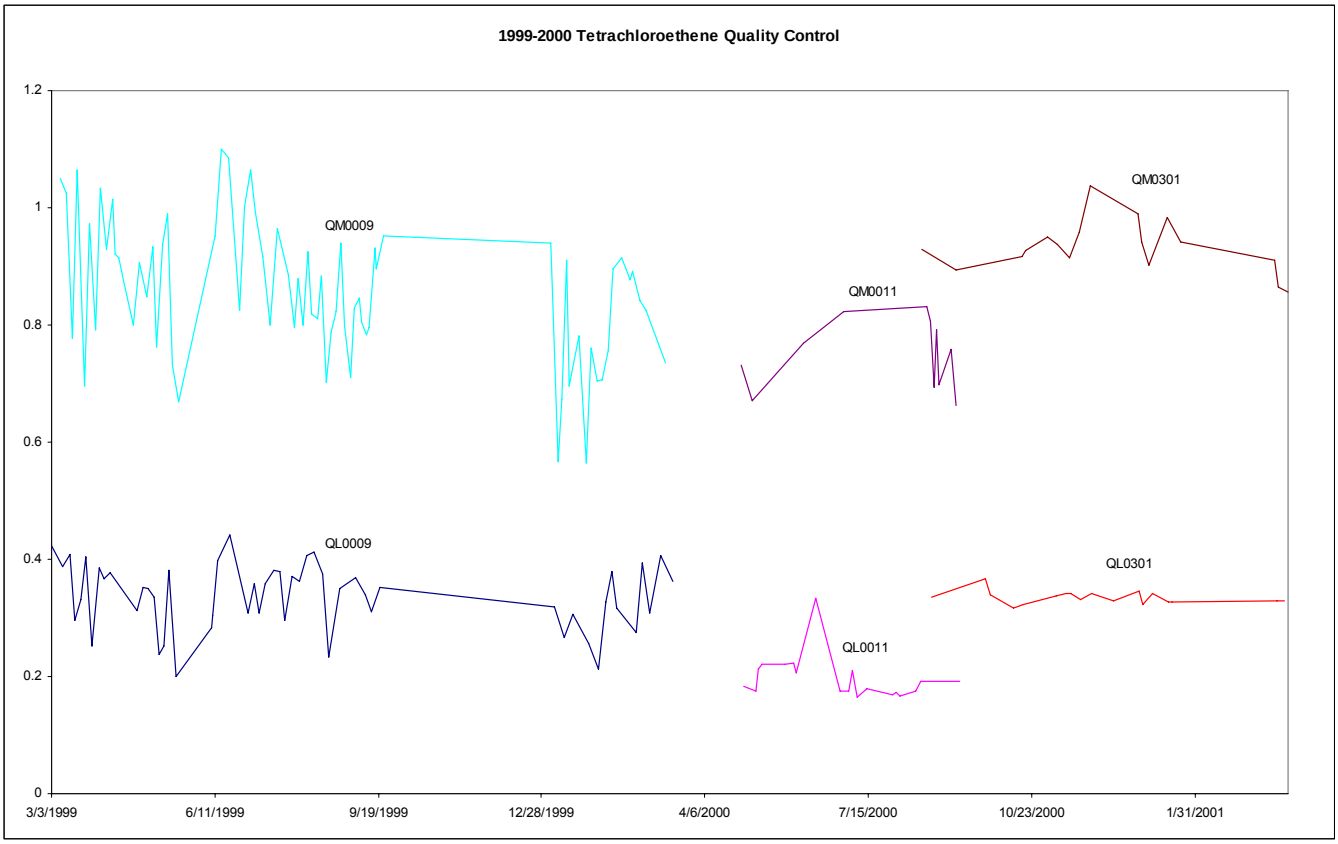
Because of the complex nature of the analyses and the unique testing capabilities of this laboratory, it is not expected that specimens will be referred to other laboratories for testing. Should such a need arise; the laboratory supervisor will consult with local subject matter experts to establish an appropriate mechanism and work process.

19. Summary Statistics and Quality Control Graphs

A. Tetrachloroethene

Summary Statistics for Tetrachloroethene by Lot

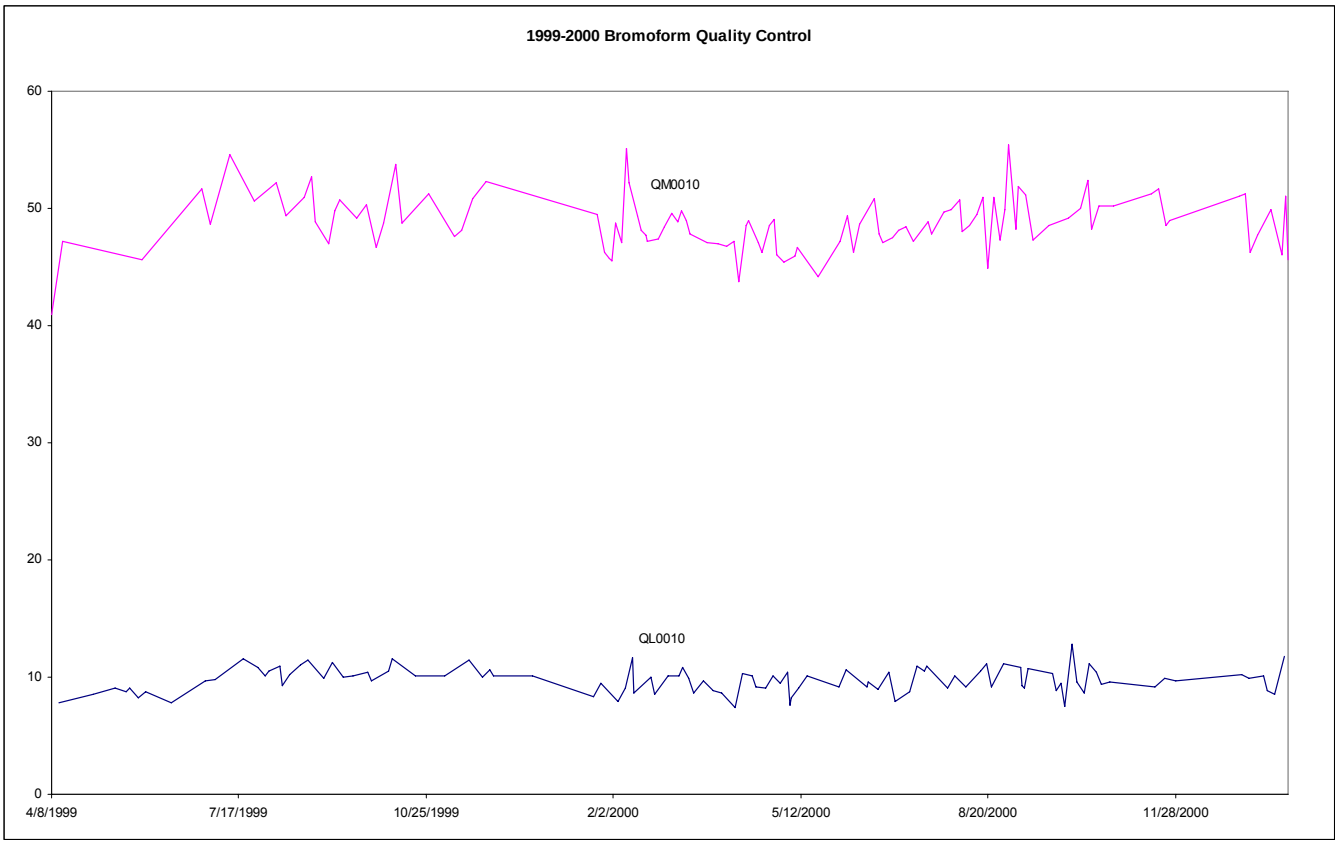
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QL0009	53	3/3/1999	3/17/2000	0.3373	0.0571	16.9
QM0009	72	3/8/1999	3/13/2000	0.8581	0.1179	13.7
QM0011	11	4/28/2000	9/7/2000	0.7485	0.0608	8.1
QL0011	19	4/30/2000	9/9/2000	0.1971	0.0384	19.5
QM0301	17	8/17/2000	3/29/2001	0.9329	0.0448	4.8
QL0301	18	8/23/2000	3/27/2001	0.3347	0.0112	3.3



B. Bromoform

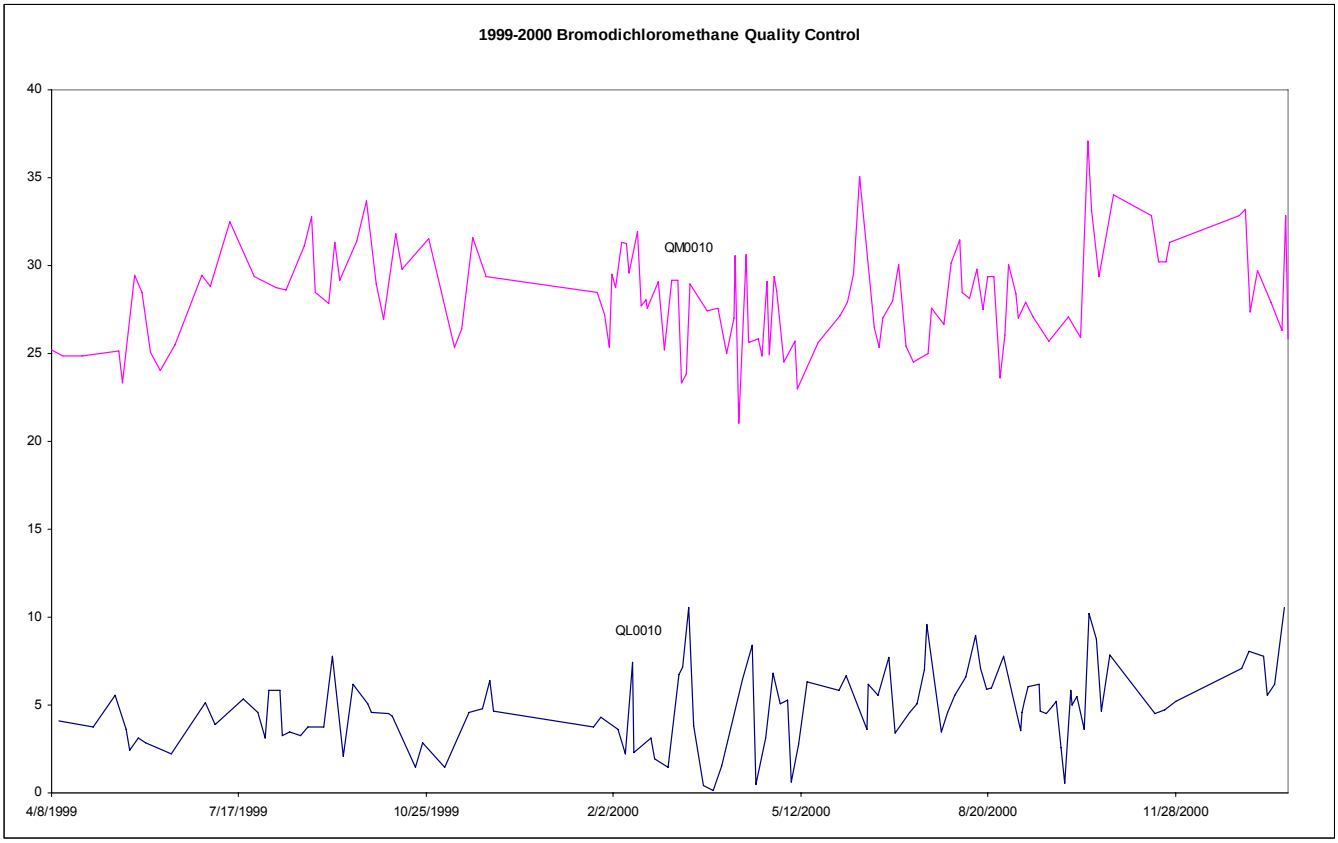
Summary Statistics for Bromoform by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QM0010	109	4/8/1999	1/27/2001	48.7852	2.3587	4.8
QL0010	105	4/12/1999	1/25/2001	9.7538	1.0451	10.7

Volatile Organic Compounds in Whole Blood
NHANES 1999-2000



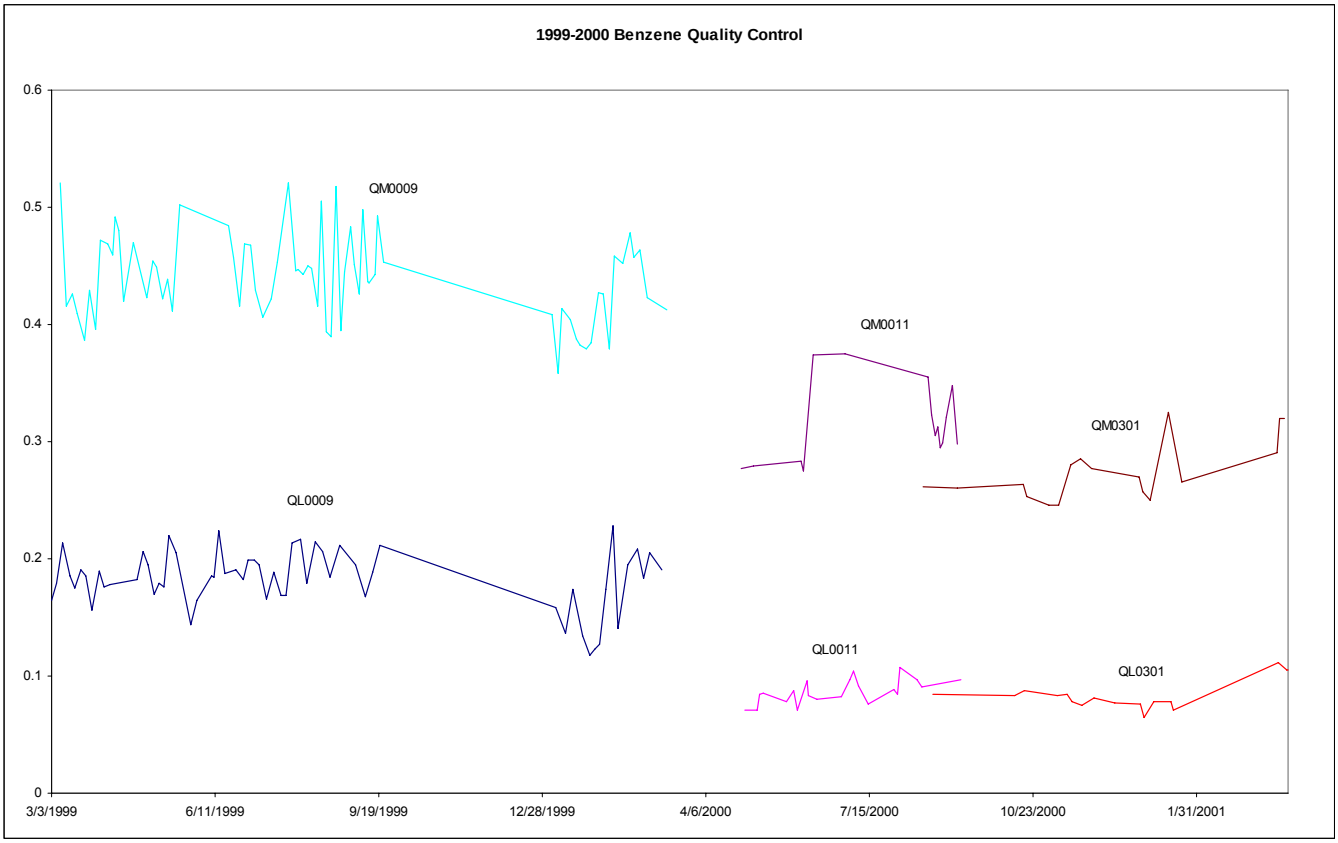
C. Bromodichloromethane

Summary Statistics for Bromodichloromethane by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QM0010	118	4/8/1999	1/27/2001	28.2684	2.8438	10.1
QL0010	108	4/12/1999	1/25/2001	4.8658	2.2082	45.4



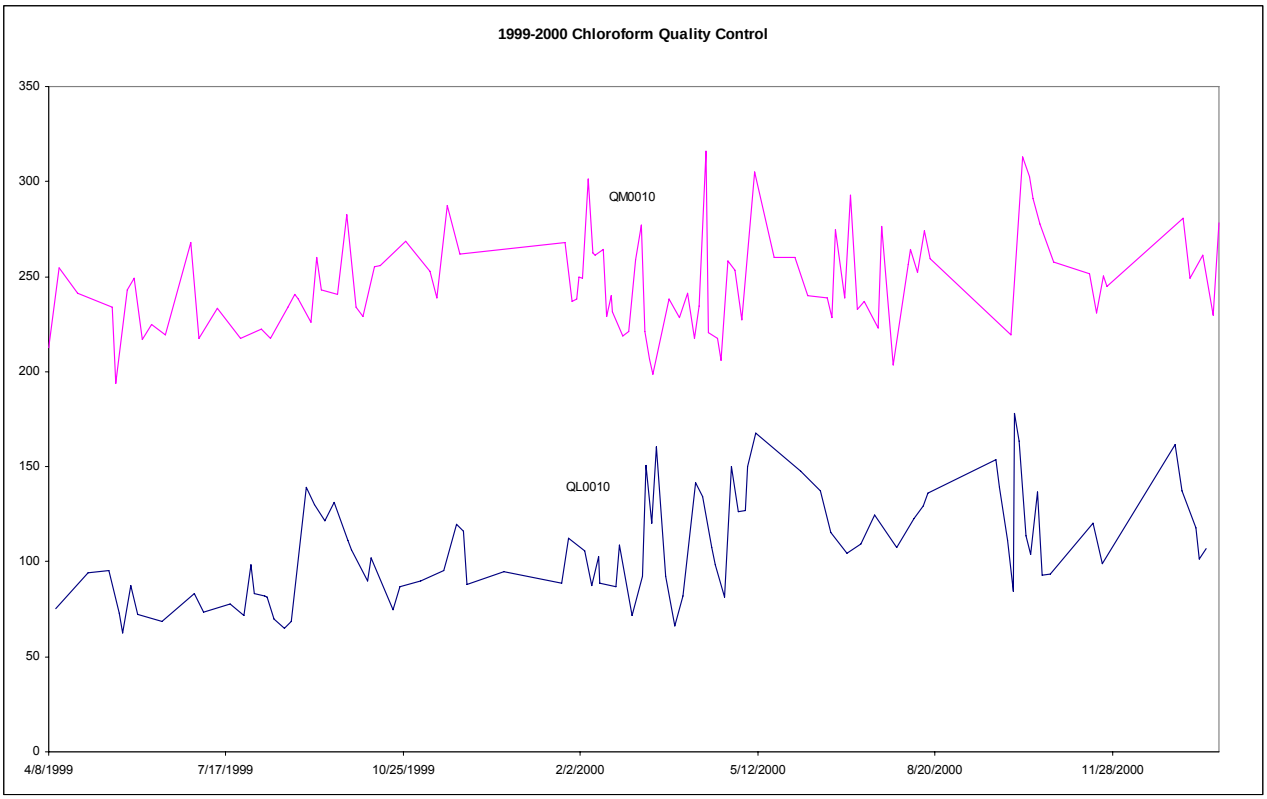
D. Benzene

Summary Statistics for Benzene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	60	3/3/1999	3/10/2000	0.1827	0.0251	13.7
QM0009	70	3/8/1999	3/13/2000	0.4397	0.0373	8.5
QM0011	15	4/28/2000	9/7/2000	0.3145	0.0341	10.8
QL0011	21	4/30/2000	9/9/2000	0.0868	0.0104	12.0
QM0301	17	8/17/2000	3/26/2001	0.2747	0.0258	9.4
QL0301	17	8/23/2000	3/28/2001	0.0837	0.0126	15.0



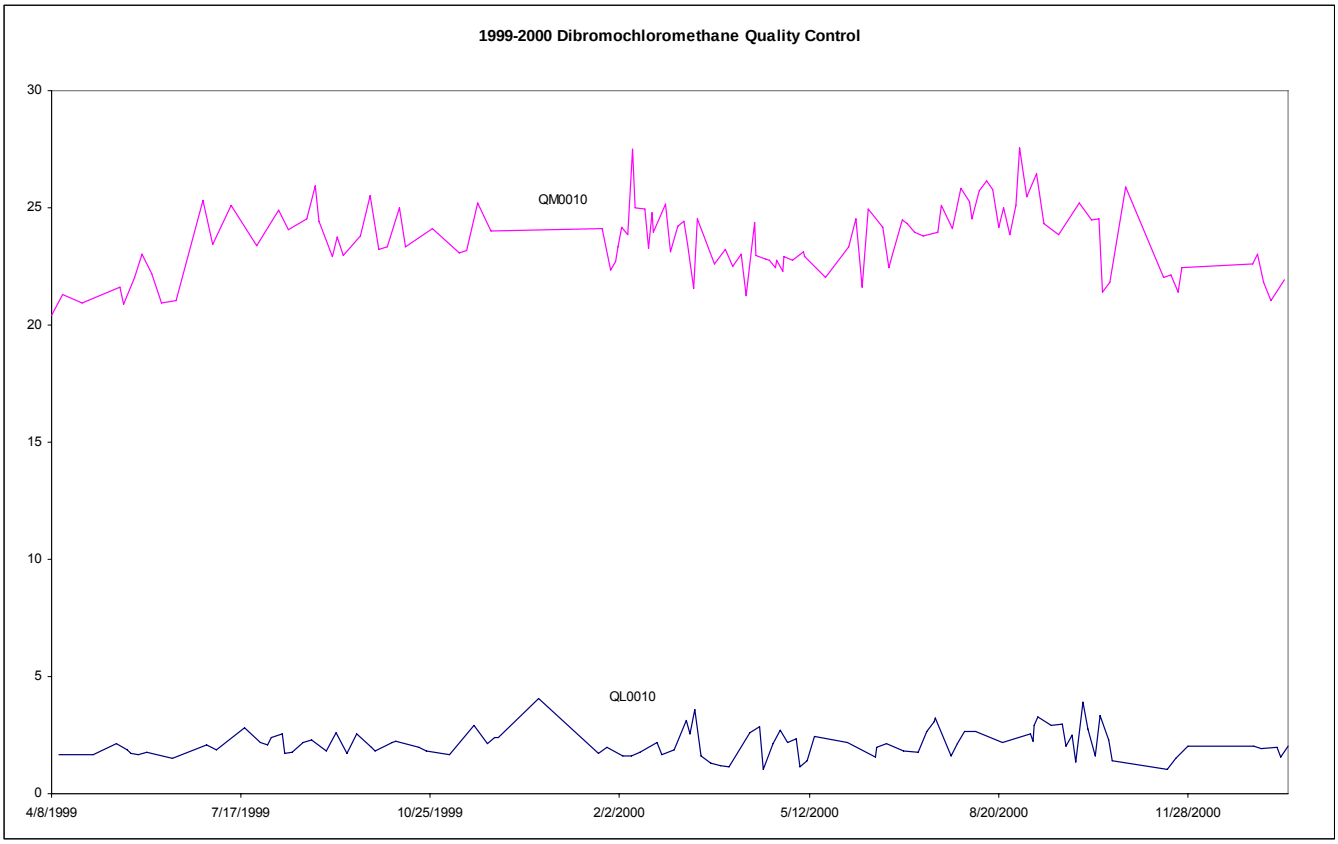
E. Chloroform

Summary Statistics for Chloroform by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QM0010	98	4/8/1999	1/27/2001	246.5675	25.6356	10.4
QL0010	89	4/12/1999	1/20/2001	107.1013	27.4657	25.6



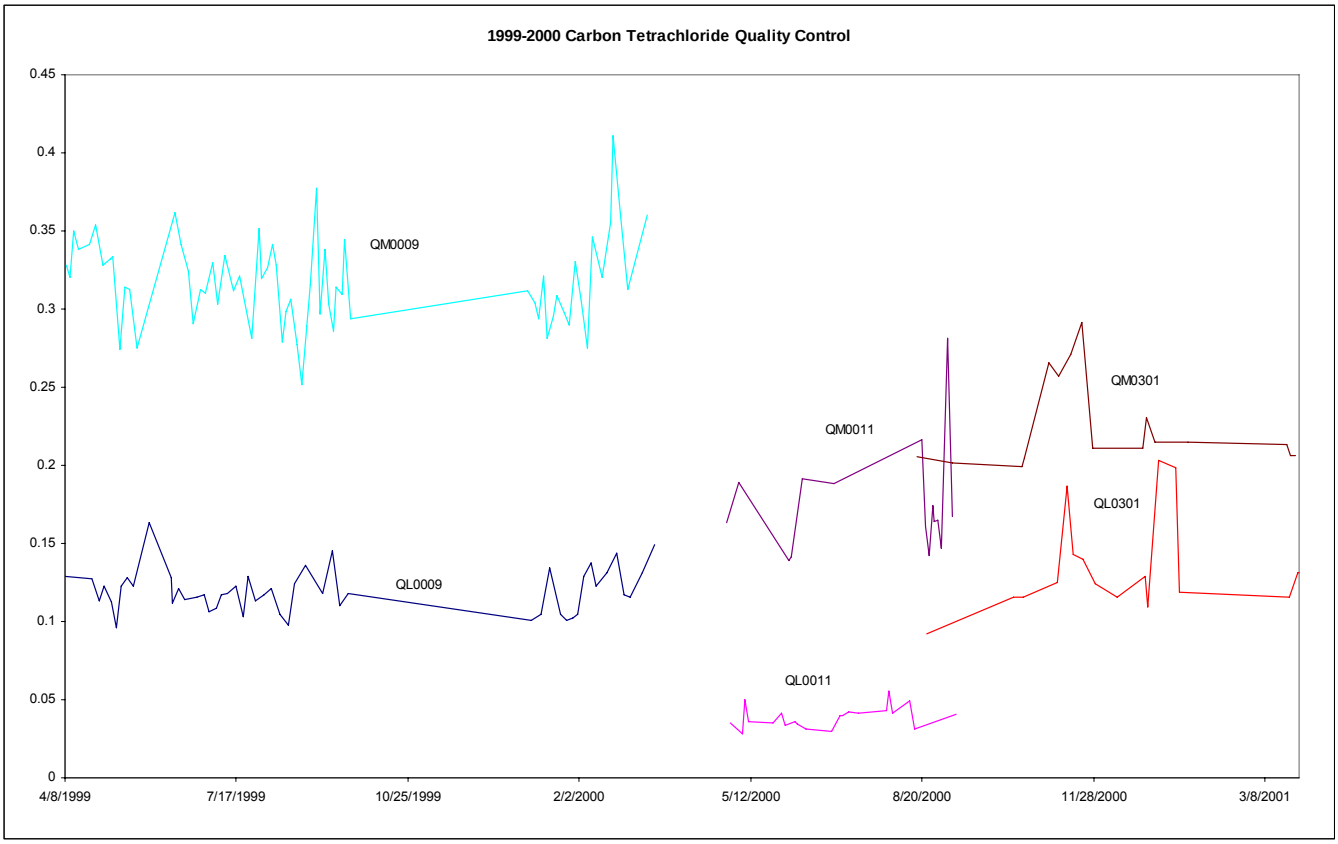
F. Dibromochloromethane

Summary Statistics for Dibromochloromethane by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QM0010	113	4/8/1999	1/18/2001	23.5998	1.4624	6.2
QL0010	97	4/12/1999	1/20/2001	2.1438	0.6059	28.3



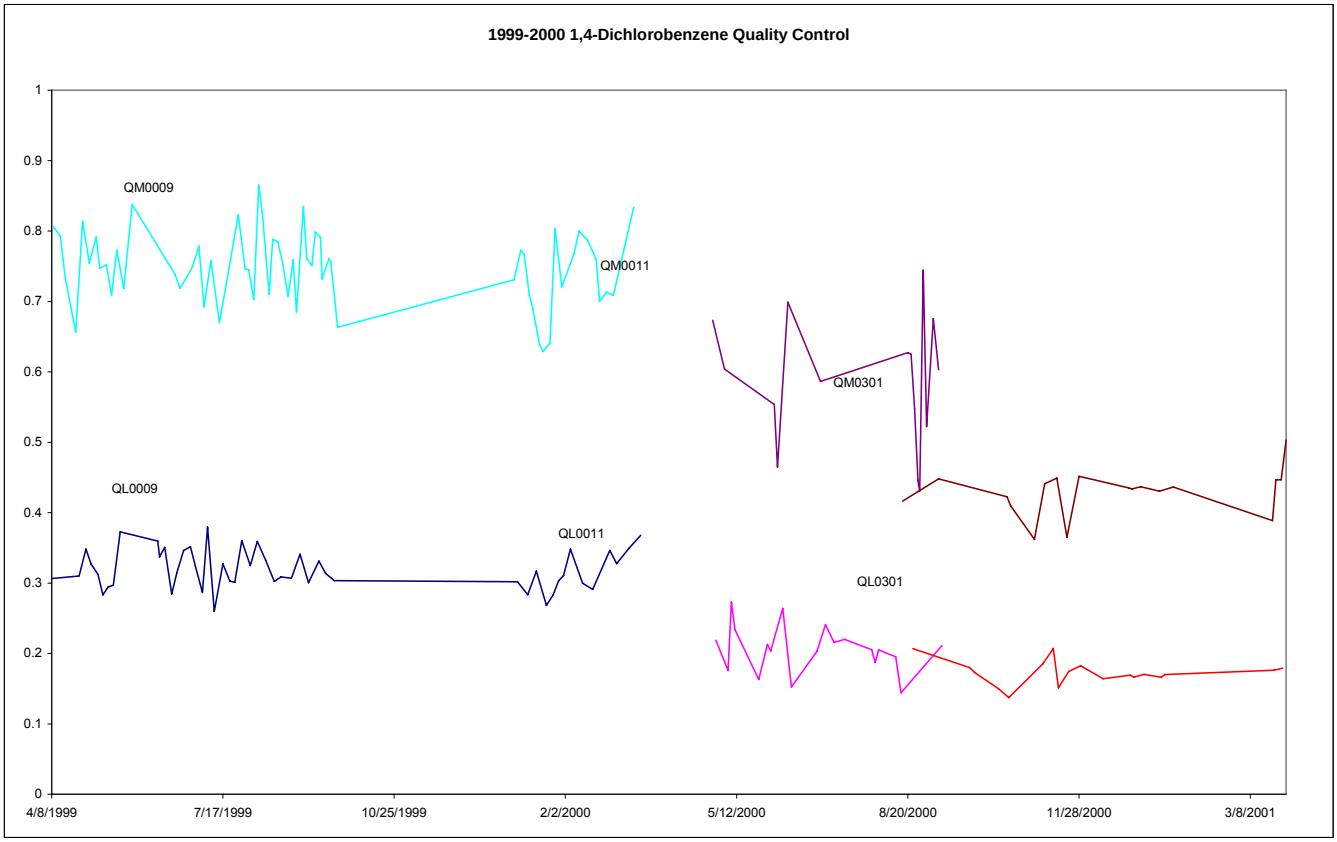
G Carbon Tetrachloride

Summary Statistics for Carbon Tetrachloride by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	50	4/8/1999	3/17/2000	0.1197	0.0139	11.6
QM0009	65	4/9/1999	3/13/2000	0.3177	0.0284	8.9
QM0011	15	4/28/2000	9/7/2000	0.1754	0.0364	20.8
QL0011	21	4/30/2000	9/9/2000	0.0388	0.0069	17.9
QM0301	16	8/17/2000	3/26/2001	0.2253	0.0290	12.9
QL0301	17	8/23/2000	3/28/2001	0.1350	0.0316	23.4



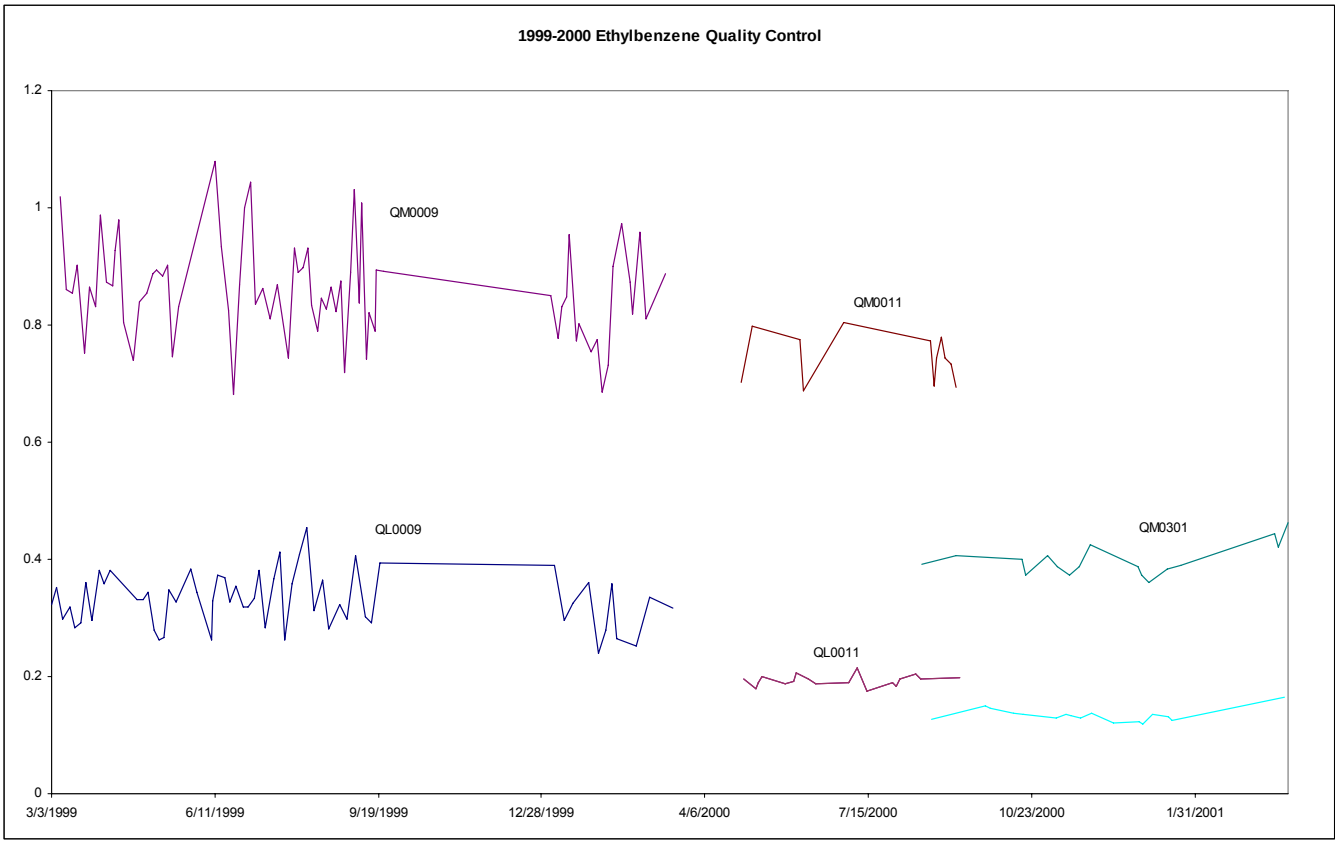
H. 1,4-Dichlorobenzene

Summary Statistics for 1,4-Dichlorobenzene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	49	4/8/1999	3/17/2000	0.3196	0.0283	8.9
QM0009	62	4/9/1999	3/13/2000	0.7479	0.0521	7.0
QM0011	15	4/28/2000	9/7/2000	0.5868	0.0934	15.9
QL0011	19	4/30/2000	9/9/2000	0.2066	0.0336	16.3
QM0301	18	8/17/2000	3/29/2001	0.4291	0.0330	7.7
QL0301	18	8/23/2000	3/27/2001	0.1728	0.0174	10.1



I. Ethylbenzene

Summary Statistics for Ethylbenzene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	58	3/3/1999	3/17/2000	0.3303	0.0462	14.0
QM0009	73	3/8/1999	3/13/2000	0.8601	0.0839	9.8
QM0011	12	4/28/2000	9/7/2000	0.7443	0.0420	5.6
QL0011	18	4/30/2000	9/9/2000	0.1933	0.0097	5.0
QM0301	17	8/17/2000	3/29/2001	0.3980	0.0268	6.7
QL0301	17	8/23/2000	3/27/2001	0.1343	0.0112	8.3

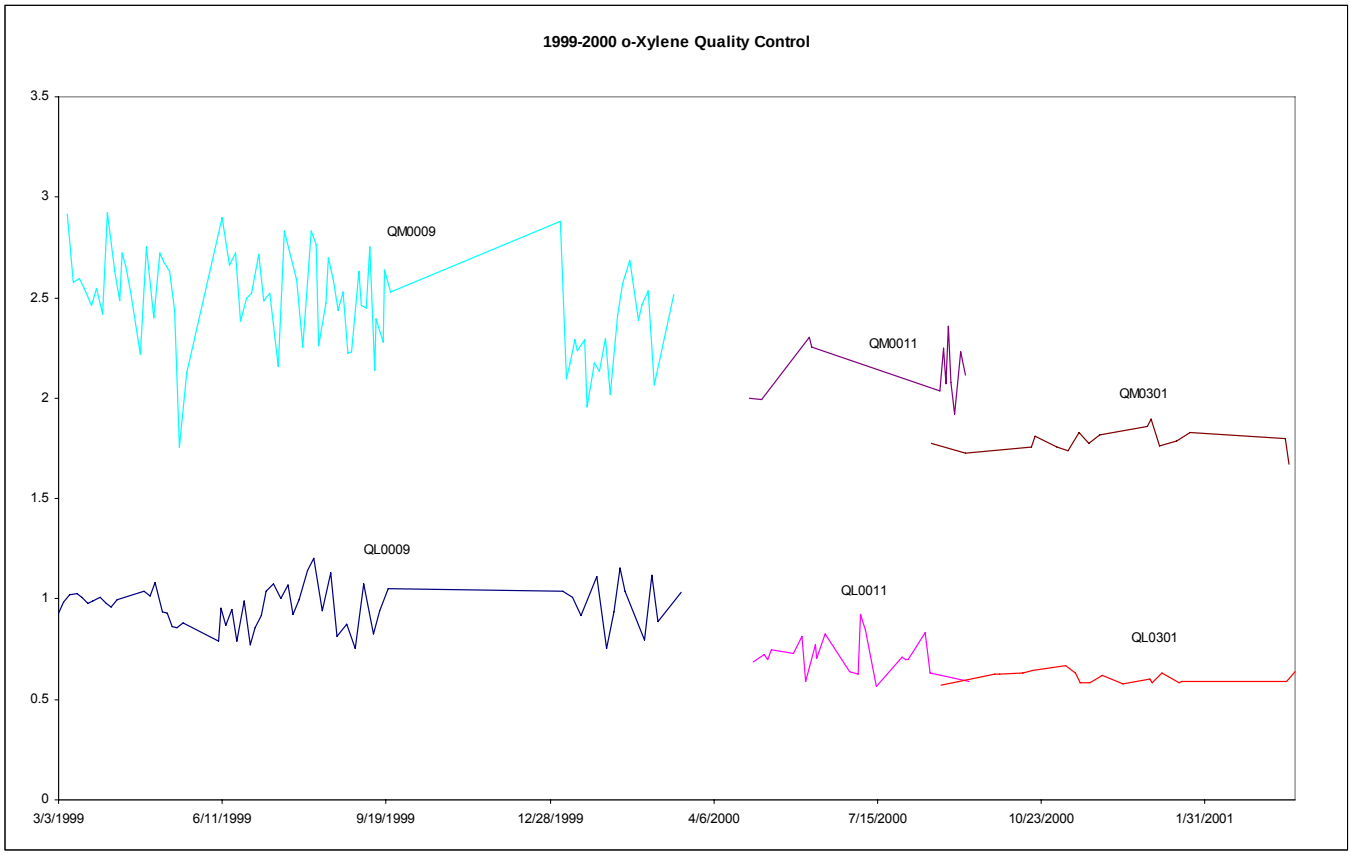


J. MTBE

Summary Statistics for Methyl t-Butyl Ether (MTBE) by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QM0010	101	6/27/1999	1/27/2001	49.6529	12.0362	24.2
QL0010	100	6/29/1999	1/25/2001	50.3969	10.4097	20.7

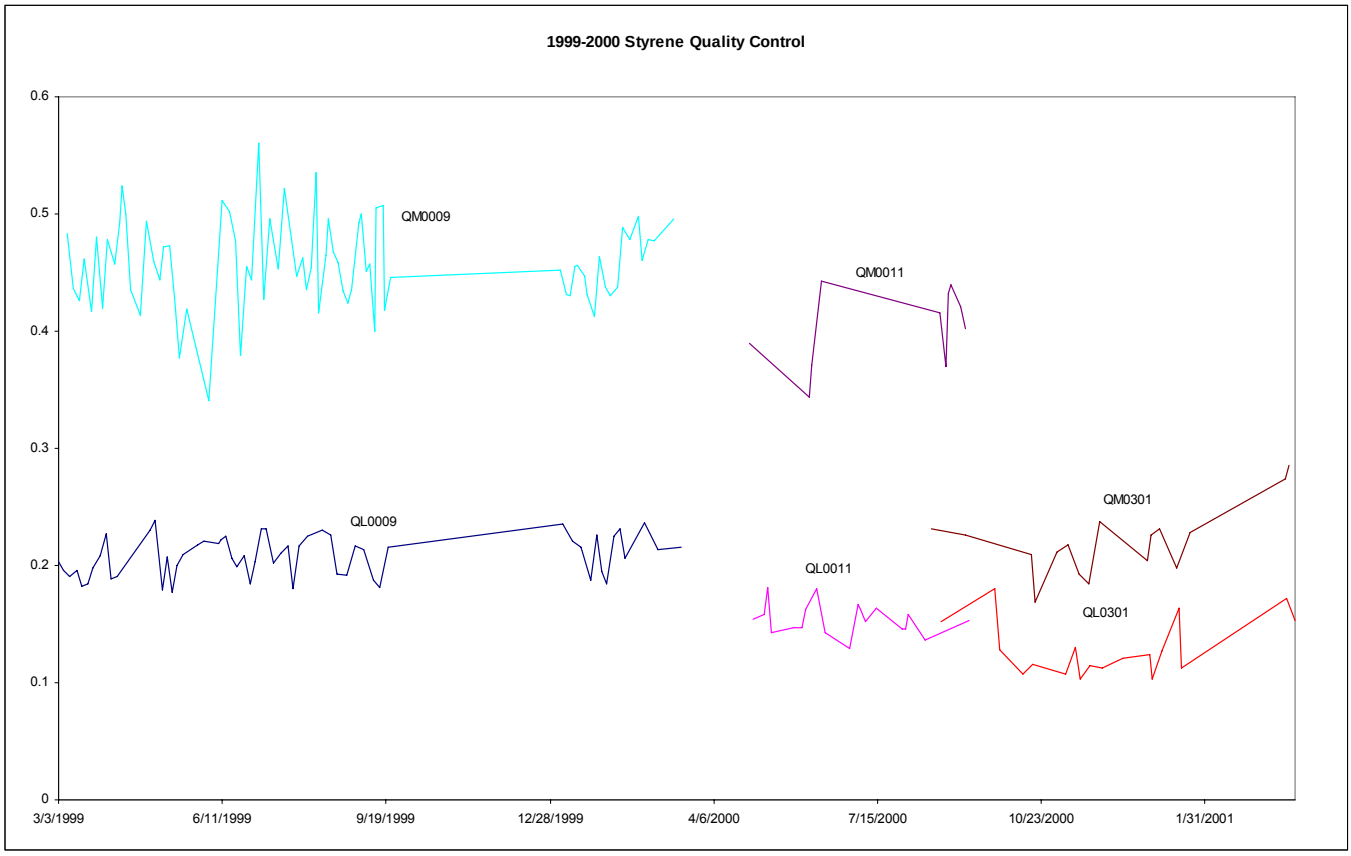
K. o-Xylene

Summary Statistics for o-Xylene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	57	3/3/1999	3/17/2000	0.9653	0.1063	11
QM0009	74	3/8/1999	3/13/2000	2.4723	0.2434	9.8
QM0011	12	4/28/2000	9/7/2000	2.1346	0.1399	6.6
QL0011	21	4/30/2000	9/9/2000	0.7165	0.0942	13.1
QM0301	16	8/17/2000	3/23/2001	1.7863	0.0536	3
QL0301	18	8/23/2000	3/27/2001	0.6101	0.0290	4.8



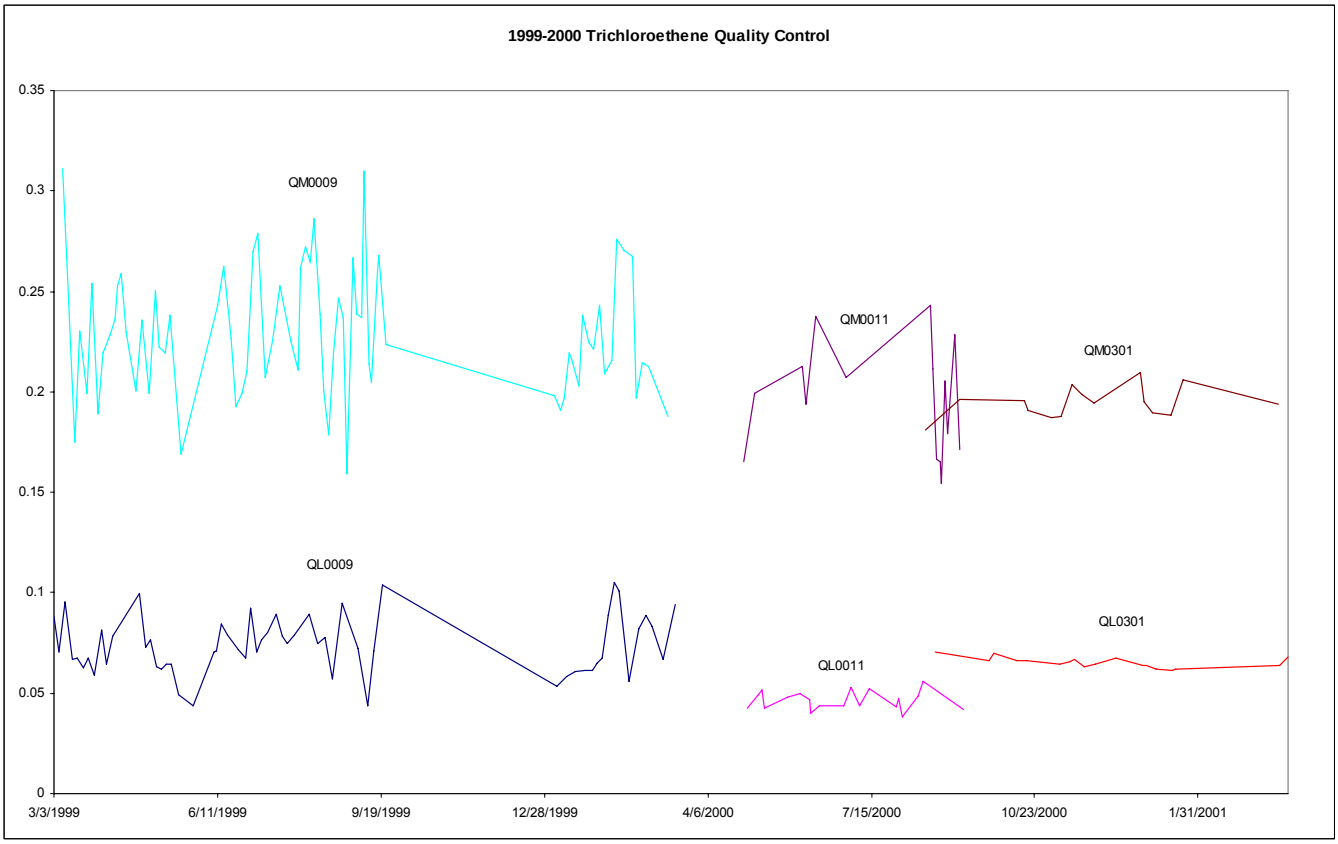
L. Styrene

Summary Statistics for Styrene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	58	3/3/1999	3/17/2000	0.2082	0.0173	8.3
QM0009	75	3/8/1999	3/13/2000	0.4577	0.0378	8.3
QM0011	10	4/28/2000	9/7/2000	0.4027	0.0335	8.3
QL0011	19	4/30/2000	9/9/2000	0.1542	0.0135	8.7
QM0301	16	8/17/2000	3/23/2001	0.2203	0.0300	13.6
QL0301	18	8/23/2000	3/27/2001	0.1292	0.0244	18.9



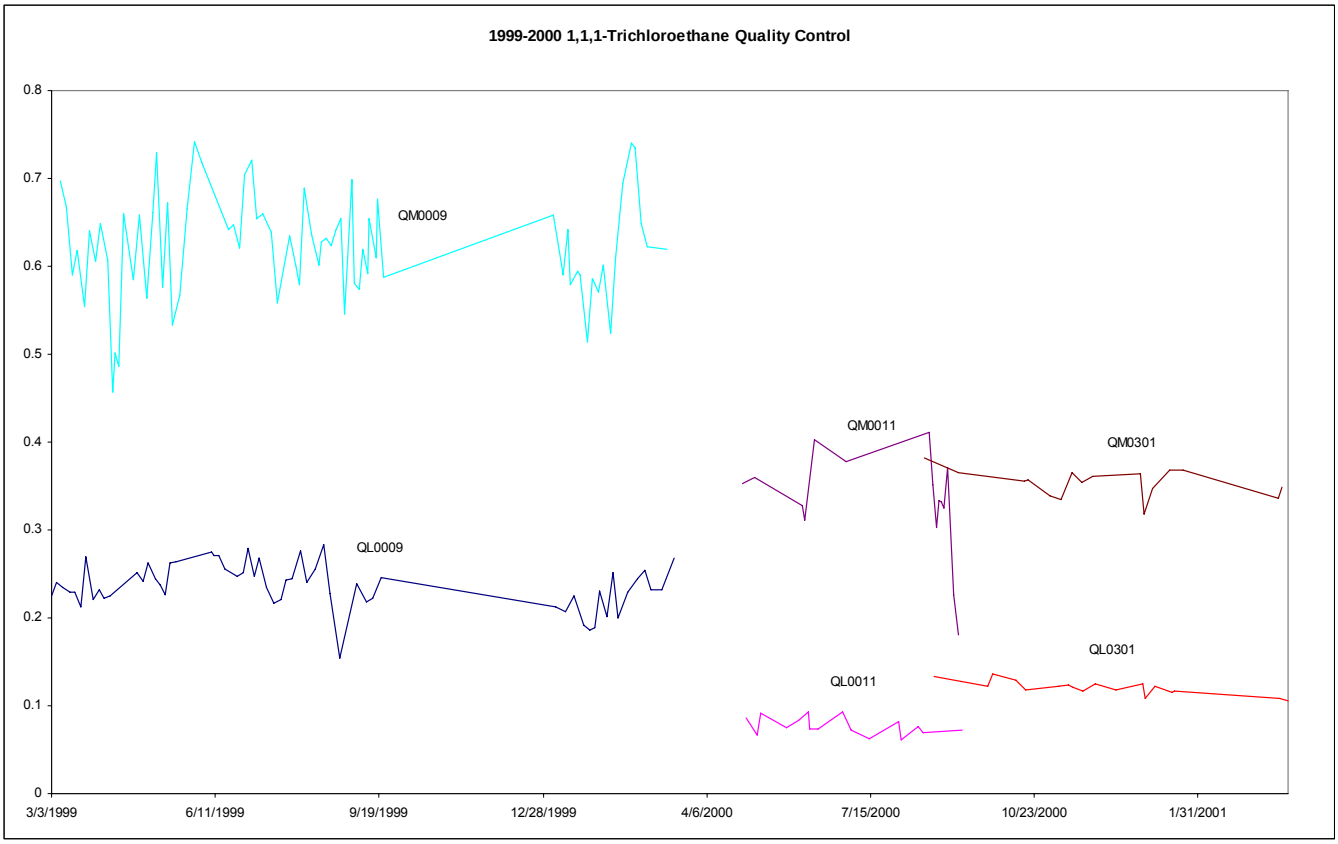
M. Trichloroethene

Summary Statistics for Trichloroethene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	59	3/3/1999	3/17/2000	0.0739	0.0144	19.4
QM0009	73	3/8/1999	3/13/2000	0.2295	0.0316	13.8
QM0011	15	4/28/2000	9/7/2000	0.1961	0.0281	14.3
QL0011	19	4/30/2000	9/9/2000	0.0464	0.0047	10.2
QM0301	15	8/17/2000	3/21/2001	0.1945	0.0077	3.9
QL0301	18	8/23/2000	3/27/2001	0.0653	0.0026	4.0



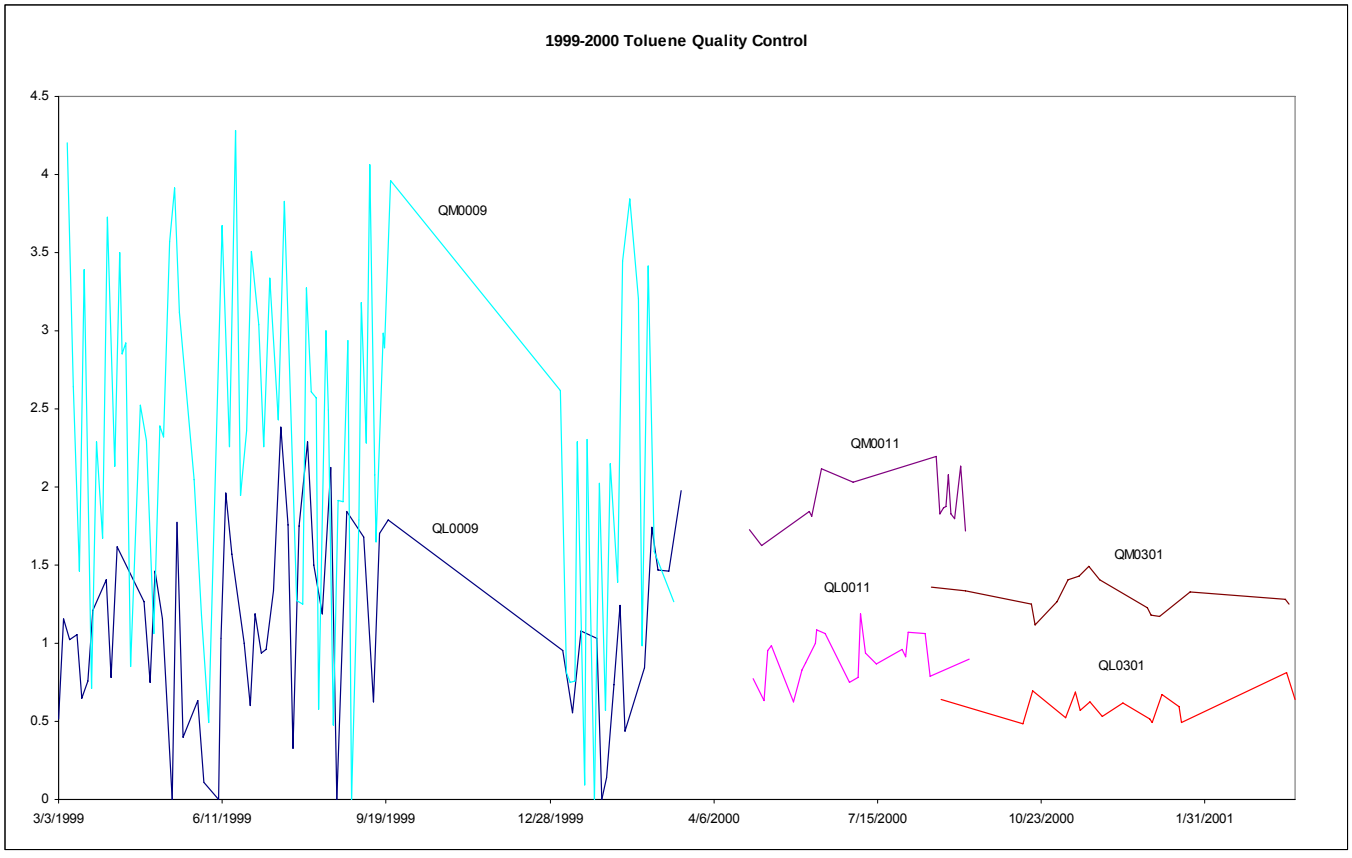
N. 1,1,1-Trichloroethene

Summary Statistics for 1,1,1-Trichloroethane by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	59	3/3/1999	3/17/2000	0.2368	0.0254	10.7
QM0009	74	3/8/1999	3/13/2000	0.6222	0.0593	9.5
QM0011	15	4/28/2000	9/7/2000	0.3311	0.0610	18.4
QL0011	16	4/30/2000	9/9/2000	0.0769	0.0103	13.4
QM0301	16	8/17/2000	3/23/2001	0.3540	0.0159	4.5
QL0301	18	8/23/2000	3/27/2001	0.1203	0.0082	6.8



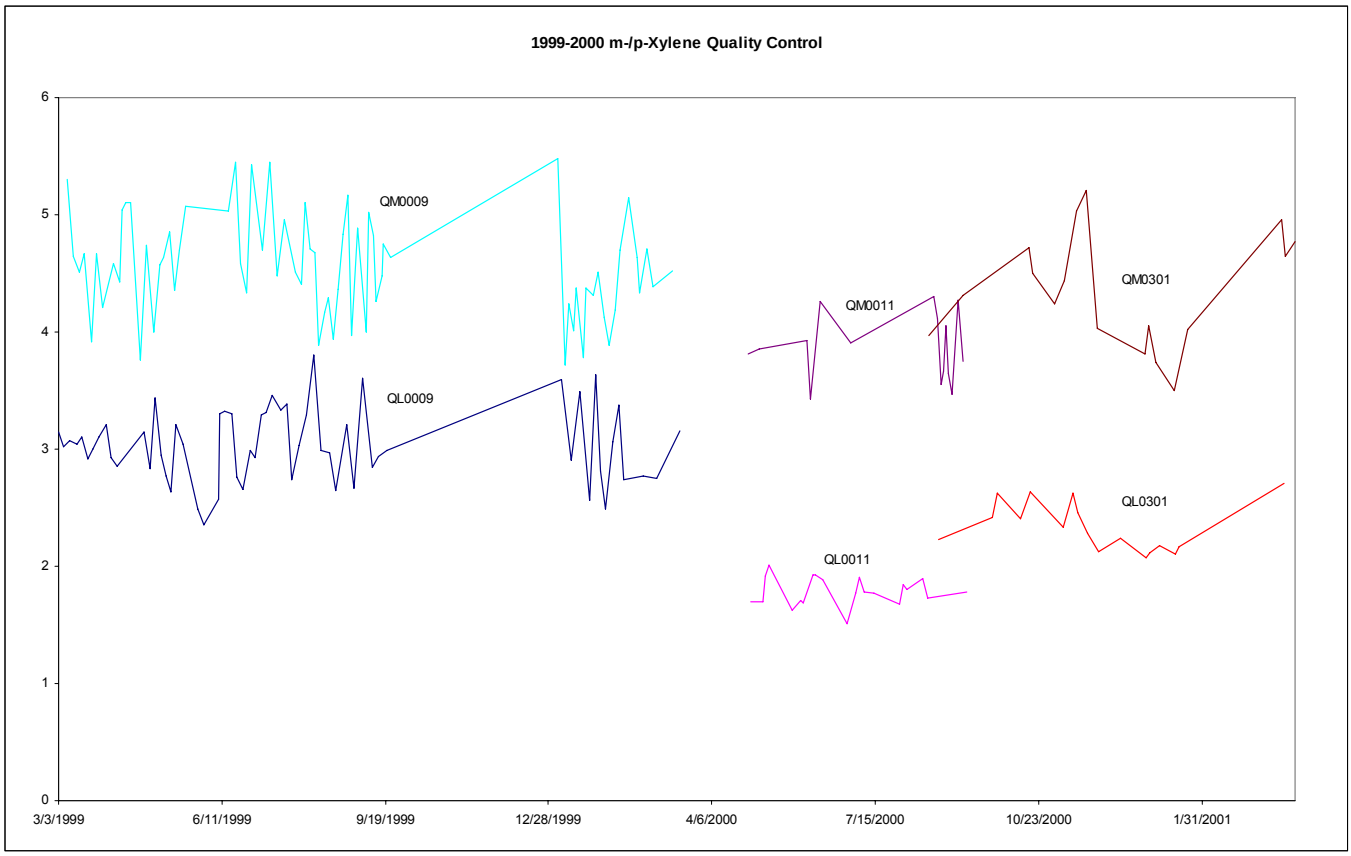
O. Toluene

Summary Statistics for Toluene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	57	3/3/1999	3/17/2000	1.1039	0.6093	55.2
QM0009	76	3/8/1999	3/13/2000	2.2739	1.1060	48.6
QM0011	15	4/28/2000	9/7/2000	1.8967	0.1701	9.0
QL0011	21	4/30/2000	9/9/2000	0.9064	0.1479	16.3
QM0301	15	8/17/2000	3/23/2001	1.3000	0.1060	8.2
QL0301	16	8/23/2000	3/27/2001	0.6003	0.0919	15.3



P. m-/p-Xylene

Summary Statistics for m-/p-Xylene by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
QL0009	60	3/3/1999	3/17/2000	3.0321	0.3152	10.4
QM0009	70	3/8/1999	3/13/2000	4.5656	0.4376	9.6
QM0011	15	4/28/2000	9/7/2000	3.8678	0.2899	7.5
QL0011	21	4/30/2000	9/9/2000	1.7881	0.1213	6.8
QM0301	17	8/17/2000	3/29/2001	4.3492	0.4903	11.3
QL0301	17	8/23/2000	3/22/2001	2.3364	0.2126	9.1



Use of trade names is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

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