Laboratory Procedure Manual

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

Matrix: **Whole Blood**

Method: **Headspace Solid-Phase Microextraction with Benchtop GC MS**

As performed by: Tobacco and Volatiles 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 testing the items listed in the following table

Data File Name	Variable Name	SAS Label
VOCWB_E	LBXV1A	Blood 1,1-dichloroethane (ng/mL)
	LBXV1D	Blood 1,2-dichlorobenzene (ng/mL)
	LBXV1E	Blood 1,1-dichloroethylene (ng/mL)
	LBXV2A	Blood 1,2-dichloroethane (ng/mL)
	LBXV2C	Blood cis-1,2-dichloroethylene (ng/mL)
	LBXV2E	Blood 1,1,2-trichloroethane (ng/mL)
	LBXV2P	Bld 1,2-dibromo-3-chloropropane (ng/mL)
	LBXV2T	Blood trans-1,2-dichloroethylene (ng/mL)
	LBXV3B	Blood 1,3-dichlorobenzene (ng/mL)
	LBXV4E	Blood 1,1,2,2-tetrachloroethane (ng/mL)
	LBXV4C	Blood tetrachloroethene (ng/mL)
	LBXV4CE	Blood 1,1,1,2-tetrachloroethane (ng/mL)
	LBXVBZ	Blood benzene (ng/mL)
	LBXVCB	Blood chlorobenzene (ng/mL)
	LBXVCT	Blood carbon tetrachloride (ng/mL)
	LBXVDB	Blood 1,4-dichlorobenzene (ng/mL)
	LBXVDE	Blood 1,2-dibromoethane (ng/ml)
	LBXVDM	Blood dibromomethane (ng/mL)
	LBXVDP	Blood 1,2-dichloropropane (ng/mL)
	LBXVEB	Blood ethylbenzene (ng/mL)
	LDXVFN	Blood furan (ng/ml)
	LBXVHE	Blood hexachloroethane (ng/mL)
	LDXVIPB	Blood isopropylbenzene (ng/ml)
	LBXVMC	Blood methylene chloride(ng/mL)
	LBXVNB	Blood nitrobenzene (ng/mL)
	LBXVOX	Blood o-xylene (ng/mL)
	LBXVST	Blood styrene (ng/mL)
	LBXVTC	Blood trichloroethene (ng/mL)
	LBXVTE	Blood 1,1,1-trichloroethane (ng/mL)
	LBXVTP	Blood 1,2,3-trichloropropane (ng/ml)
	LBXVTO	Blood toluene (ng/mL)
	LBXVXY	Blood m-/p-xylene (ng/mL)
	LBX2DF	Blood 2,5-dimethylfuran (ng/mL)

1. Clinical Relevance and Summary of Test Principle

a. Clinical Relevance

Biomonitoring of volatile organic compounds (VOCs) in blood provides useful information on exposure and internal dose of environmental chemicals. To support studies exploring the relationship between exposure to these chemicals and adverse health effects, an automated analytical method was developed using capillary gas chromatography (GC) and mass spectrometry (MS) with selected-ion monitoring (SIM) detection and isotope-dilution. This method quantifies levels of individual VOCs in blood to low-parts-per-trillion range. Because nonoccupationally exposed individuals have blood VOC concentrations in this range, this method is applicable for determining these quantities and investigating cases of sustained or recent low-level exposure.

b. Test Principle

Volatile organic compounds are measured in specially collected whole blood samples by headspace solid-phase microextraction (SPME)/gas chromatography/isotope dilution mass spectrometry using a similar method as described by Blount, *et al*.[1] Analysis of the blood sample is performed by equilibrium headspace analysis using SPME. For analysis, 3-ml of blood is transferred by gas-tight syringe from a blood collection tube to a headspace vial. The SPME fiber is inserted into the headspace of a hermetically sealed sample vial containing the blood sample. The VOCs partitions into the coating on the outside of the SPME fiber shaft. This fiber is then inserted into the heated GC inlet where the VOCs rapidly desorb because of the high 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 SIM MS (unit mass resolution). Response calibration is performed using isotopically labeled standards to normalize calibration standards and blood sample responses. This method is applicable to the determination of a broad range of VOCs in 3-ml blood with detection limits in the low-parts-per-trillion range. Because nonoccupationally exposed individuals have blood VOC concentrations in this range, this method is applicable for determining these quantities and investigating cases of exposure to VOCs.

Alteration of particular aspects of this method can result in major biases. Care is required to produce non-contaminated blanks, blood collection tubes, and quality control materials. Efforts must be taken to minimize the sources of VOC contamination. Some typical contamination sources include the use of solvents, bleach and cleaning products, dry-cleaned clothing, air fresheners, perfumes, fuel/exhaust fumes, off-gassing from paints/adhesives/plastics, and inadequate lab air handling.[2-4]

2. Computerization; Data-System Management

a. Software and knowledge requirements

Data are processed and reviewed with instrument software provided by the instrument manufacturer. The reviewed data are entered into a relational database.

b. Sample information

Information pertaining to particular specimens is entered into a database either manually or electronically transferred. Blood samples from each analysis batch are processed into a single file using data analysis software that provides blood level results along with

corresponding calibration curve, QC, blank data. 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 involves visual inspection of all peak integrations, proofreading all transcribed data, storage of data in multiple computer systems, and redundant data archiving. Original data files contain traceable header information (e.g., date, analytical run number, sample type and sample identification) and are stored on recordable media on site. Data is accessed directly by Ethernet connection to the instrument computer. 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 database system using an automated data import module.

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, password and/or smart card 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).

3. Procedures for Collecting, Storing, and Handling Specimens; 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 of the analytical measurement. Contamination is prevented by drying the site that has been swabbed with isopropyl alcohol with a gauze bandage and allowing it to dry for 5 to 10 sec.

The specimen type is whole blood collected in specially cleaned, 10-ml or 7-ml draw glass tubes containing potassium oxalate and sodium fluoride anticoagulant. Additional information on preparation of these blood collection tubes can be found in Section 6.d.

c. Sample handling

The CDC-prepared blood collection tubes contain anticoagulant that inhibits metabolism and prevents coagulation. Metabolic inhibition increases sample shelf life by minimizing metabolic impact on blood VOC levels during storage. Once samples have been collected, they are mixed thoroughly to completely dissolve and distribute the anticoagulant. Because blood is perishable and VOCs are highly volatile, care is taken to insure that samples are kept at refrigerator temperatures (i.e., 2–6ºC) during storage and

shipment. All samples are placed on wet ice or into a refrigerator within 30 min of sample collection. In addition, samples are shipped with enough wet ice or equivalent cooling material to insure that the samples remain cool (but not frozen) throughout the shipment process. Shipment is scheduled to ensure that they will arrive at CDC on normal business days to guarantee their proper processing upon arrival. Samples are not frozen or stored at freezer temperatures at any time during sample collection and shipment. Samples are shipped within 1 to 2 days of collection so that they are typically 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. After 10 weeks of storage the blood often begins to thicken and is therefore difficult to handle. Even though analytical results may not change over this time, samples may be less amenable to analysis. Certain volatile organic compounds are produced naturally, and metabolism may alter their concentration with storage.

Storage at freezing temperatures results in cell rupture. In addition, freezing of blood can lead to breakage of blood collection tubes and loss of sample in some cases. Because VOCs are lost whenever the containers in which they are contained are opened, blood samples are not transferred to another container.

d. Sample quantity

The blood collection tube is filled to capacity to minimize headspace losses. Headspace losses depend upon the blood-air partition constant of a compound. The minimum acceptable amount for analysis 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 causing the blood sample to clot, suspected contamination, use of an untreated blood collection tube, and significant clotting of the specimen.

Failure to obtain adequate sample volume is obvious when the samples are received. Visual inspection of the blood collection tube reveals when 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 blood collection tube is inverted. Clotting can occur from failure to properly mix the sample and anticoagulant as described above. A description of reasons for each rejected sample is recorded in the relational database as the samples are logged into the laboratory.

4. Preparation of Reagents, Calibration Materials, Control Materials, and All Other Materials; Equipment and Instrumentation

a. Reagents and sources

1) Solvents

Solvents and how they are used are listed below;

<u>HPLC grade acetone</u> is sometimes used for primary dilution of neat native standards and labeled analogs for improved solubility of nonpolar compounds. Before use, HPLC

grade acetone is verified through analytical measurement not to significantly bias the analytical measurement.

Purge and trap grade methanol is used for all intermediate native standards and isotopically labeled internal standards. Before use, purge and trap grade methanol must first be shown not to significantly bias the analytical measurement.

HPLC grade water is primarily used to produce VOC free water. Variability in contaminant levels requires testing of product lots. This water is further processed by nitrogen sparging and distillation to further reduce VOCs before use. Methods for this procedure are based on previously published techniques for removing residual VOCs from reagent water.[5] Water is validated to contain no detectable levels of those VOCs being analyzed.

2) Calibration and Control Materials

Compounds used for preparation of calibration standards and quality control materials are listed in Table 1 and are purchased from companies meeting guidelines of International Organization of Standards Guide 34. Recommended isotopically labeled internal standards listed in Table 2 can be used. Other isotopic analogs may be used because of availability and costs limitations as long as there are no interfering chromatographic or mass spectral interferences. All chemicals are used without further purification unless required. Native standard materials are at least 97% pure. Isotopically labeled internal standards are of sufficient chemical and isotopic purity to produce levels needed for accurate quantitation and impurities do not interfere with analyses of the other VOC analytes.

Table 1. Reagents for calibration and control materials

Compound	Formula	Safety
1,1,1,2-Tetrachloroethane	$CHCl_3CHCl$	a,b,e
1,1,1-Trichloroethane	CH_3CCl_3	a,b
1,1,2,2-Tetrachloroethane	$CHCl_2CHCl_2$	a,c
1,1,2-Trichloroethane	$CH_2ClCHCl_2$	a,b
1,1-Dichloroethane	CH_3CHCl_2	a,b
1,1-Dichloroethylene	$CH_2{=}CCl_2$	c,d
1,2,3-Trichloropropane	$ClCH_2CH(Cl)CH_2Cl$	a,b,e
1,2-Dibromo-3-chloropropane	$^{13}CH_2Br^{13}CHBr^{13}CH_2Cl$	b,d
1,2-dibromoethane	$BrCH_2CH_2Br$	a,b,e
1,2-Dichlorobenzene	$C_6H_4Cl_2$	b,e
1,2-Dichloroethane	CH_2ClCH_2Cl	a,d
1,2-Dichloropropane	$CH_3CHClCH_2Cl$	d,e
1,3-Dichlorobenzene	$C_6H_4Cl_2$	b,e
1,4-Dichlorobenzene	$C_6H_4Cl_2$	a,b
2,5-Dimethylfuran	$C_6H_{12}O$	b,d
Benzene	C_6H_6	a,d
Carbon Tetrachloride	CCl_4	a,b
Chlorobenzene	C_6H_5Cl	d,e
cis/trans-1,2-Dichloroethylene	$CHCl{=}CHCl$	d,f
Dibromomethane	CH_2Br_2	a,b,e
Ethylbenzene	$C_6H_5CH_2CH_3$	d,e
Furan	C_4H_4O	a,b,d,e
Hexachloroethane	$ClC(Cl_2)CCl_3$	a,e
Isopropylbenzene	$C_6H_5CH(CH_2)_2$	b,d,e
m/*p*-Xylene	$C_6H_4(CH_3)_2$	d,e
Methylene Chloride	CH_2Cl_2	b,e
Nitrobenzene	$C_6H_5NO_2$	b,d
o-Xylene	$C_6H_4(CH_3)_2$	d,e
Styrene	$C_6H_5CH{=}CH_2$	a,d
Tetrachloroethylene	$CCl_2{=}CCl_2$	a,g
Toluene	$C_6H_5CH_3$	b,d
Trichloroethylene	$CHCl{=}CCl_2$	a,g

a - Cancer suspect agent b – Toxic c – Lachrymator
d – Flammable e – Irritant f - Moisture sensitive
g – Mutagen

Table 2. Isotopically labeled internal standards

Compound	Formula	Safety
1,1,1,2-Tetrachloroethane-$^{2}H_2$	$C^{2}HCl_3C^{2}HCl$	a,b,e
1,1,1-Trichloroethane-$^{2}H_3$	$C^{2}H_3CCl_3$	a,b
1,1,2,2-Tetrachloroethane-$^{2}H_2$	$C^{2}HCl_2C^{2}HCl_2$	a,c
1,1,2-Trichloroethane-$^{2}H_3$	$C^{2}H_2ClC^{2}HCl_2$	a,b
1,1-Dichloroethane-$^{2}H_3$	$C^{2}H_3CHCl_2$	a,b
1,1-Dichloroethylene-$^{2}H_2$	$C^{2}H_2{=}CCl_2$	c,d
1,2,3-Trichloropropane-$^{2}H_5$	$ClC^{2}H_2C^{2}H(Cl)C^{2}H_2Cl$	a,b,e
1,2-Dibromo-3-chloropropane-$^{13}C_3$	$^{13}CH_2Br^{13}CHBr^{13}CH_2Cl$	b,d
1,2-dibromoethane-^{c}C	$Br^{13}CH_2CH_2Br$	a,b,e
1,2-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	b,e
1,2-Dichloroethane-$^{2}H_4$	$C^{2}H_2ClC^{2}H_2Cl$	a,d
1,2-Dichloropropane-$^{2}H_6$	$C^{2}H_3C^{2}HClC^{2}H_2Cl$	d,e
1,3-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	b,e
1,4-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	a,b
2,5-Dimethylfuran-$^{13}C_2$	$(^{13}CH_3)_2C_4H_6O$	b,d
Benzene-$^{13}C_6$	$^{13}C_6H_6$	a,d
Carbon Tetrachloride-$^{13}C_1$	$^{13}CCl_4$	a,b
Chlorobenzene-$^{13}C_6$	$^{13}C_6H_5Cl$	d,e
cis/trans-1,2-Dichloroethylene-$^{2}H_2$	$C^{2}HCl{=}C^{2}HCl$	d,f
Dibromomethane-$^{2}H_2$	$C^{2}H_2Br_2$	a,b,e
Ethylbenzene-$^{13}C_6$	$^{13}C_6H_5CH_2CH_3$	d,e
Furan-$^{2}H_4$	$C_4{}^{2}H_4O$	a,b,d,e
Hexachloroethane-$^{13}C_1$	$^{13}CCl_3CCl_3$	a,e
Isopropylbenzene-$^{2}H_5$	$C_6{}^{2}H_5CH(CH_2)_2$	b,d,e
m-/p-Xylene-$^{13}C_6$	$^{13}C_6H_4(CH_3)_2$	d,e
Methylene Chloride-$^{13}C_1$	$^{13}CH_2Cl_2$	b,e
Nitrobenzene-$^{13}C_6$	$^{13}C_6H_5NO_2$	b,d
o-Xylene-$^{2}H_6$	$C_6H_4(C^{2}H_3)_2$	d,e
Styrene-$^{13}C_6$	$^{13}C_6H_5CH{=}CH_2$	a,d
Tetrachloroethylene-$^{13}C_1$	$^{13}CCl_2{=}CCl_2$	a,g
Toluene-$^{13}C_7$	$^{13}C_6H_5{}^{13}CH_3$	b,d
Trichloroethylene-$^{13}C_1$	$^{13}CHCl{=}CCl_2$	a,g

a - Cancer suspect agent b – Toxic c – Lachrymator
d – Flammable e – Irritant f - Moisture sensitive
g – Mutagen

b. Preparation of glassware

Glassware that is found to have detectable levels of VOC residue is solvent rinsed and vacuum baked to ensure removal of contamination for all VOCs being analyzed. All glassware is kept in a vacuum oven at sufficient temperature and pressure to prevent VOC recontamination. Before use, the glassware is cooled to room temperature under vacuum. When glassware is removed from the oven it is sealed with polytetrafluoroethylene (PTFE) lined caps, when appropriate.

c. Preparation of headspace vial septa

Headspace vial septa are verified to provide sufficient seal to maintain detection above the limit of detection. Septa are cleaned by vacuum baking and verified through analysis of the bulk material to be free of those VOCs being analyzed.[3] After cleaning, septa are stored in a vacuum oven of sufficient vacuum and temperature to prevent VOC recontamination.

d. Preparation of blood collection tubes

Blood collection tubes (e.g., Vacutainers) obtained from commercial sources contain high levels of VOC residue in the butyl rubber stoppers. This residue can mask the levels of VOC analytes originally in the blood at the time of sample collection, and thus prevent accurate exposure assessment. The most commonly encountered blood collection tube VOC contaminants are listed in Table 3. Blood collection tube lot variation is avoided by purchasing in batches of 10,000, which lasts several years. The selected lot is prescreened for VOC residue levels. To prevent sample contamination, the VOCs are removed from blood collection tubes using a special cleaning method.[6] A combination of solvent swelling and vacuum baking is used to remove residue levels from the rubber stopper that interfere with accurate quantitation.[4] Following treatment of the blood collection tubes, the tubes are labeled with a new expiration date that reflects a 1-year shelf life. The shelf life of a blood collection tubes is limited mainly by the amount of time the blood collection tube can remain under vacuum, however VOC residue levels from the stopper can increase with time if deeply penetrated VOCs are not completely removed. Tubes used are supplied by laboratory sciences (DLS) staff for all blood VOC studies.

Table 3. Analytes most commonly found to contaminate blood collection tubes

Analyte
1,1,1-Trichloroethane
1,4-Dichlorobenzene
Benzene
Dibromomethane
Ethylbenzene
4-Methyl-2-pentanone
m/*p*-Xylene
o-Xylene
Styrene
Tetrachloroethylene
Toluene
Trichloroethylene

Blood collection tube rubber stoppers are cleaned by removing the stoppers from the glass section of the tube. Stoppers are solvent swelled in boiling purge and trap grade methanol and vacuum baked for at least 3 weeks. The glass tube and anticoagulant salt are vacuum baked to remove any adsorbed residue. Once cleaned, the blood collection tubes are reassembled and reevacuated through a needle.

Cleaned blood collection tubes are tested for acceptable vacuum with a water-draw volume check. A sample of at least 5 tubes per batch of 300 is evaluated to ensure that contamination is below detectable levels for those analytes being analyzed. For this evaluation VOC free water is stored in the blood collection tubes oriented horizontally for 7–14 days at room temperature and then analyzed.

e. Preparation of blank water

Distilling, dispensing and storing of water are performed to minimize contamination from the surrounding environment and validated by comparison with an established reference

or by standard addition. HPLC grade water is used as the starting material. The HPLC grade water is cleaned by heated sparging with nitrogen, boiling and refluxing, hermetic dispensing into precleaned ampules (cleaned in accordance with 4.b), and flame sealing with a water torch. After production, the flame seal is verified to be leak tight.

f. Preparation of native analytical standards

1) Handling of neat compounds

Inexpensive analytes purchased as neat liquids in flame sealed ampules are discarded after use. Expensive compounds (e.g., custom synthesis products) are saved for future use in flame sealed ampules. All compounds stored for future use that are expected to exceed the manufacturer's expiration date must be stored in an explosion-proof -70ºC freezer. However, purity must be revalidated by quantitation upon reuse. Short-term storage of neat standards that are not to exceed the manufacturers expiration date are stored at 2–6ºC in a chemical storage refrigerator separate from blood samples, blanks and quality control materials.

2) Filling and sealing of glass ampules

Neat standard materials to be stored for reuse are transferred to glass ampules, typically 1 ml, and filled under three-quarters capacity. Ampules are chilled throughout the aliquoting process. Pipettes are conditioned with the material before transferred to the storage ampules. Liquid is placed in the bottom of the ampule and is not adhering to the neck of the ampule before flame sealing. Sealed ampules are leak checked.

3) Final concentrations of the standards

Standards are formulated starting with the primary stock solutions prepared from neat materials diluted with either purge and trap grade methanol or HPLC acetone. Lower concentration primary stock solutions involve only a single serial dilution of the highest concentration stock. Seven intermediate levels are formulated from the primary stock solutions in purge and trap grade methanol using only a single dilution step. The 7 working standards are prepared in VOC free water using a single dilution of the corresponding intermediate levels and are separated by a factor of $\sqrt{10}$ ranging from low ppt to low ppb levels. The water is verified to have VOCs below detectable levels for the analytes of interest. Positive displacement pipettes are used for transfer of all liquids in the µl range with at least 2% accuracy. Class A volumetric flasks are used to make all standards. The primary stock solution concentrations are based on the gravimetric measure of mass transferred to the volumetric flask.

4) Aqueous working standards

Aqueous working standards are formulated in 25-ml quantities with added internal standard. 3.0 ml of each the aqueous working standards is transferred into cleaned 10-ml headspace vials using a gas-tight glass barrel/PTFE plunger pipetter. The vials are immediately sealed with recently cleaned caps and grouped by concentration in separate wide mouth specimen jars to prevent cross contamination. Furthermore, the standard set is stored in a dedicated refrigerator at 2–6ºC and analyzed as part of an analytical batch within 1 week.

g. Preparation of isotopically labeled internal standard solutions

1) Primary isotopically labeled internal standard stock solutions

Primary isotopically labeled internal standard stock solutions are made by dilution of the neat compound into purge and trap grade methanol. These solutions are stored in 1-ml ampules and flame sealed as described in Section 6.f.2. Concentrations of the primary labeled internal standard stock solutions are analyte dependent and range from 0.5 to 20 mg/ml. The primary isotopically labeled internal standard stock solutions are stored in a freezer below -60ºC.

2) Secondary isotopically labeled internal standard stock solutions

The secondary isotopically labeled internal standard stock solution is made by combining primary stock solutions and diluting to concentrations between standard levels 2 and 5. Solutions are mixed thoroughly and approximately 0.25 ml of these solutions are flame sealed in chilled 1-ml ampules as described above in Section 6.f.2. Ampulized stock solutions are stored in a freezer below -60ºC.

3) Working isotopically labeled internal standard solution

The working isotopically labeled internal standard solution is prepared daily from the ampulized secondary stock solution. The secondary stock solution is added to the standard formulations, water blanks, QC samples and unknown samples proportionally. Final labeled internal standard concentrations in samples vary depending on the analyte and are less than standard level 5 but greater than standard level 2. For storage the working solution is transferred directly from the formulation flask to cleaned 2-ml screw cap vials inset with PTFE lined septa. Vials are filled to leave no headspace and stored at 4ºC no longer than 2 days.

h. Preparation of quality control materials

QC materials are prepared at two concentration levels in fetal bovine serum. Residue is removed from fetal bovine serum by either sorption or volatilization under hermetic conditions; however, certain compounds are difficult to remove while maintaining the properties of the serum. Target and measured concentrations vary because of significant background levels that might not be removed during the cleaning process or adsorption/diffusion loss during sample preparation. The characterized mean is determined by analysis of at least 20 separate samples using different sample runs and instruments. The low concentration QC material is typically between standard level 1 and 3, whereas the high concentration QC material is between standard level 3 and 5. The QC materials are thoroughly mixed under hermetic conditions, transferred and flame sealed in 10-ml ampules for storage below -60ºC. Short-term storage up to -20°C may occur but does not exceed 6 weeks. The concentration consistency across the lot is evaluated by comparing samples prepared at the beginning, middle and end of the batch. If this comparison revealed variability of more than 25% for any analyte, the lot was reformulated.

i. Proficiency testing materials

Proficiency testing (PT) materials are prepared at four levels from neat compounds in a manner similar to the intermediate standard materials. PT intermediate solutions are prepared, aliquoted into ampules, and flame sealed using the same preparation technique as described in Section 6.f.2. The PT reference materials are purchased from a different

International Organization of Standards Guide 34 certified chemical company than those used in the formulation of the standards. PT solutions are prepared at intermediate concentrations and diluted in the same manner as the standards. Each of the 4 final PT concentrations lies between standard levels 2 and 3, levels 3 and 4, levels 5 and 6, and levels 6 and 7. A proficiency testing coordinator, independent from the sample analysis team, blind-codes the PT stock ampules and administers and verifies accuracy of quantified results of 4 PT samples at each of the 4 concentration levels and one sample at any of the 4 different levels.

j. Clean-up procedure for the 5-ml Luerlock gas-tight syringe

Each blood sample is delivered from the blood collection tube to the headspace vial using a cleaned 5-ml Luerlock gas-tight syringe. Headspace syringes are decontaminated with a 10% bleach solution and thoroughly cleaned of VOC residue with rinses of filtered deionized water and purge and trap grade methanol followed by vacuum baking. Syringe cleaning batches are verified to be clean by analysis of 2 water control samples prepared using two randomly selected syringes with each sample run.

k. Instrumentation and operation

SPME of the headspace sample is performed using a Combi-Pal autosampler. Samples are queued on an autosampler tray and maintained at 15±0.5ºC until they are analyzed. During analysis the samples are transferred to an agitating incubator set to at least 350 rpm and 40±1ºC as the headspace is sampled with a 75-µm Carboxen-PDMS coated SPME fiber (Supelco, Bellefonte PA) for consistently specified time period of at least 6 min and no more than 15 min. The SPME fiber is then immediately transferred into the GC injection port fitted with a glass liner with an i.d. of 1 to 2 mm and held at 250±0.5ºC. The sample is introduced into an Agilent DB-VRX column (40m x 0.18mm x 1µm film) via pulsed splitless injection set at 50 psi. After 1.0 min, but no more than 2 min, the injection port pressure is then dropped to maintain a constant flow of 1.1±0.1 ml/min of helium. In-line after the injection port is a cryogenic trap. At the start of the GC run the cryotrap is set to approximately -100ºC for at least 1 min, but no more than 2 min, then ballistically heated to approximately 225ºC (13.0ºC/sec). The GC oven temperature is programmed to ramp from 0ºC (1.5 min hold) at 7ºC/min to 140ºC, then 40ºC/min to 220ºC (for at least a 4.5 min hold). Quantitation by a quadrupole MS is performed using SIM of each primary quantitation ion, confirmation ion, and internal standard ion using at least a 15-ms dwell time for each. Examples of possible ions are shown in Table 4. When required, qualitative analyses are performed using full mass scan from m/z 21 to 500. Identification of

unknowns is established by comparison of GC retention time with that of a known standard and mass spectral data.

Table 4. Example of ions used for the internal standard and the quantification and confirmation of compounds, listed in elution order

Compound	ISTD (m/z)	Native (m/z)	Confirm (m/z)
Furan	72	68	39
1,1-Dichloroethylene	100	96	98
Methylene chloride	85	84	49
Trans-1,2-dichloroethylene	100	96	98
1,1-Dichloroethane	66	63	65
cis-1,2-dichloroethylene	100	96	98
1,2-Dichloroethane	67	62	64
1,1,1-Trichloroethane	102	97	99
Carbon tetrachloride	120	117	119
Benzene	84	78	77
Dibromomethane	178	174	93
1,2-dichloropropane	116	71	70
Trichloroethylene	133	130	132
2,5-Dimethylfuran	98	95	96
1,1,2-trichloroethane	102	97	83
Toluene	98	91	92
1,2-dibromoethane	111	107	109
Tetrachloroethylene	169	166	164
1,1,1,2-Tetrachloroethane	137	131	133
Chlorobenzene	118	112	77
Ethylbenzene	97	91	106
m/p-Xylene	97	91	106
Styrene	110	104	103
1,1,2,2-tetrachloroethane	137	131	133
O-Xylene	112	91	106
1,2,3-Trichloropropane	116	110	75
Isopropylbenzene	110	105	120
1,3-dichlorobenzene	152	146	148
1,4-dichlorobenzene	152	146	148
1,2-dichlorobenzene	152	146	148
Hexachloroethane	204	201	166
Nitrobenzene	129	123	77

Sample queues run for extended time periods of up to 24 hr. All samples awaiting analysis are racked into chilled trays (15±1°C). If the measurement is delayed to the next day, samples are left on a cooled sample tray at 15±1°C. Samples are not placed in a refrigerator that has not been recently vented.

The SPME fiber assembly can entrap VOCs and is evaluated before use. Typically, a conditioned SPME fiber is baked out in the GC inlet at 250°C for a minimum of 5 hours before the VOC contaminants fully partition out of the fiber assembly. A fiber blank, prepared by injecting the fiber without sampling a vial, is evaluated using the same analytical GCMS method as an unknown to confirm that all VOC concentrations are below instrument background levels. During the analytical run, the SPME fiber remains in the

GC injection port until ready to collect the next sample and is not exposed to the laboratory air for more than 1 min so as to reduce the influence of ambient contamination.

The analysis of VOCs in whole blood at parts-per-trillion levels is an extremely complex measurement. There are no alternative analysis approaches that achieve the combined sensitivity and specificity over the broad range of compounds described in this method. At times when the analytical system fails, the prepared samples are stored between 2 and 15°C for no more than 48 hr before analysis.

5. Calibration and Calibration Verification

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

a. Instrument response calibration

A full set of 7 calibrators is analyzed with each batch of data and used for the quantitation of analytes in all samples from that batch. Calibration curves are constructed for each analyte from the response ratios of the 7 calibrators, which are separated in concentration by a factor of $\sqrt{10}$. The slope and intercept of curves are determined by linear least squares of data weighted 1/X. Calibration curves for some compounds can be linearized by universal transform by adjusting for background and internal standard ion contribution to the standard ion and/or exclusion of nonlinear portion of the curve. A non-linear curve can be fit with a second order quadratic curve as long as no data points are quantified through extrapolation. Calibration curves are composed of at least 5 standard levels that span the range of all detectable unknown samples, and achieve a squared coefficient of determination of at least 0.98. 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.

b. Calibration verification

Calibration accuracy is tested with each run by analysis of water blank and quality control samples. A full set of calibrators is analyzed with each batch of blood samples. Absolute accuracy is verified by performance tests at approximately 6 months.

6. Procedure Operation Instructions; Calculations; Interpretation of Results

a. Analysis of samples

Prior to analysis, all blood and QC samples are thoroughly mixed and equilibrated at room temperature. For analysis, 3-ml samples of blood, bovine serum QC, and water blank samples are transferred to standard 10-ml headspace vials via separate 5-ml Luerlock gas-tight syringes fitted with disposable 18 gauge needles. Each sample is immediately spiked with the working internal standard solution, which is delivered by positive displacement pipette, and capped. Sample quantities are verified gravimetrically. All materials have been cleaned in accordance with methods described in Section 6.

b. Data analysis

Samples are quantified by their analyte ion peak area (or peak height) to internal standard ion peak area (or peak height) ratio, which compensates for loss after sample preparation, as well as variation in partitioning and SPME extraction efficiency. Blood, water blank and QC sample concentrations are multiplied by the appropriate dilution factor, which are determined by sample weight, and quantified from the standard response curves.

c. Data Processing

1) Peak Integration

Each peak is visually inspected and peak integration is corrected if the integrator erroneously integrates a peak. The integration approach for all samples is kept consistent for a specific target ion. However, if the absolute ion signal is not at least a factor of 3 above the peak-to-peak noise then peak integration is not adjusted or values are not reported. For levels measured above the LOD, confirmation ion signal is quantified.

2) Excluding calibrators

Calibrator data is only excluded if the data significantly affects (>10%) the detectable result and the cause affecting only that standard is identified. Scenarios that might only affect a single standard include a poor seal on a headspace vial, a cracked secondary standard ampule, no or low addition of internal standard, and contamination of the standard set during storage. However, standards level 6 and/or 7 can be excluded if the calibration curve is nonlinear over this region and all QCs and unknowns fall below standard level 5.

3) Excluding sample data

Sample data is excluded if no or low ISTD is added to the blood sample. Absolute internal standard response is evaluated for consistency among the standards, water blanks, QCs and blood samples. An unusually high internal standard level can occur if the ISTD is added twice. A low or absent ISTD response can occur if no internal standard is added, an intermediate standard vial was cracked, or a vial cap seal was poor.

d. Formal Quality Control Material Evaluation

Quality control sample results are formally evaluated by an independent quality control officer following import of data into a relational database. The QC samples analyzed with a batch of data are evaluated against the characterized 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.[7,9] 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.

e. 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, sufficient internal standard response, and water blank sample bias.

f. Blood sample repeat limits

Unknown blood samples that yield concentrations higher than the 95th 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 measurements of samples stored at 2–6°C indicate that whole blood VOC samples can be banked for a total of 16 weeks if the blood remains unclotted and homogenized. Because these are whole blood samples, longer storage results in samples which are harder to manipulate, which produces additional analytical problems. Thus, even though analytical results may not change over this time, samples may be less amenable to analysis.

7. Reportable Range of Results

a. Reportable Limits

The lowest reportable value is the higher of the detection limit and the lowest standard. The upper reportable value is the highest linear standard.

b. Limits of Detection

Limits of detection are based on calculation of three times the standard deviation at zero concentration ($3S_0$) as described by Taylor.[7] Assay detection limits change with improvements in sensitivity, precision, and sample integrity.

8. Quality Assessment

Quality assurance and quality control procedures follow standard practices.[8] Daily experimental checks are made on the stability of the analytical system. Standards and quality control materials are added to each day's run sequence. At least three quality assessment sample types are analyzed in each run that include the water blank and two QC samples at different concentrations. All of these samples are prepared with the unknown blood samples. In addition to these samples, there is a water blank prepared with the standards. Absolute responses and their retention times from the lowest calibrator are evaluated from the previous run to verify method and instrument performance.

a. Accuracy

Absolute accuracy is evaluated by blind analysis of independently prepared certified proficiency test (PT) materials. Certified standard reference materials from the National Institute of Standards & Technology (NIST) are the first choice for independent validation of method accuracy. However, NIST only certifies a few of the VOCs that are measured. For example, NIST does not produce a reference standard for benzene. Because the issue of NIST traceability has long been recognized as a limitation with organic standards, we use reference materials from companies who are accredited with International Organization for Standardization (ISO) Guide 34 certification. Guide 34 is recognized by NIST, the international reference material body and other government agencies. PT samples and calibration standards are checked against the currently validated reference standard set. If a concentration for a compound in the PT sample or calibration standard is found to differ by more than 15% from the reference standard, then a validation

standard containing the compound in question along with a control compound is formulated using a NIST or Guide 34 material to verify and correct the inaccuracy.

The PT results are evaluated by a PT Coordinator. Five PT samples are analyzed twice a year using the same method described for unknown samples. The analysis passes proficiency testing if > 80% of the results deviate < 25% from the known value. If an analyte fails to meet these minimum PT criteria, it fails the test. Blood sample results are not reported for those analytes that do not successfully pass proficiency testing.

Accuracy is also verified by spiked recovery from blood. Because blood is an unstable matrix, no standard reference material is available. Thus, it is necessary to prepare these samples in-house using the intermediate standard solutions. The accuracy basis for this method is established by determining the recovery of spiked blood samples. The percent recoveries fall between 75 and 125%.

Relative accuracy is evaluated upon comparison of characterized QC mean values with those obtained on each run. Error in relative accuracy should not exceed the precision of the characterized QC samples. If such error occurs, the source of error is identified and the data corresponding to the analyte with the failed QC is not reported.

b. Precision

Precision is evaluated using the QC sample results. Two different pools of quality control material are used, one at a low and the other at a high concentration. Expected precision ranges for the QC samples are established for a new QC batch by performing at least 20 separate analyses extending over different samples, batches, days, and instruments. One instrument characterizes no more than 2 samples from one ampule per day. 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. Relative standard deviations for the QC samples are in most cases less than 15%. Standard deviations are larger for analytes with high background levels in the bovine serum.

c. Analytical Specificity

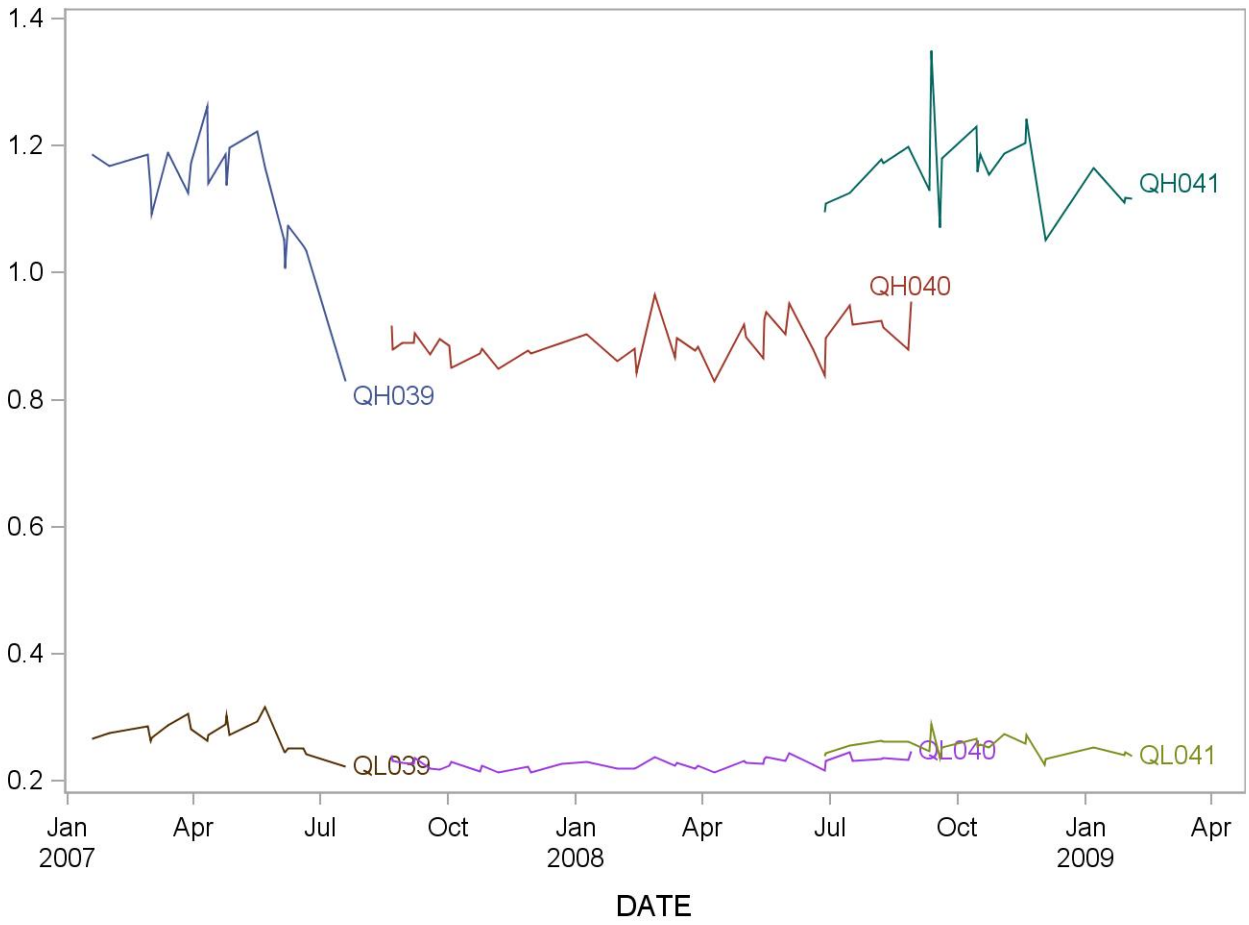
Analytical specificity is established by comparing the ratios of the areas (or height) of analyte ion chromatographic peaks with those of confirmation ions along with GC retention times relative to the ISTD ion.

Additional steps taken to achieve analytical specificity involves removing interfering compounds from the sample analysis system. Interferences that have their source in the measurement apparatus itself are examined by measuring instrument blanks. All materials and reagents used for this assay are screened and treated to remove possible interferences as described above in Section 4. The presence of co-eluting interferences is monitored by using water blanks.

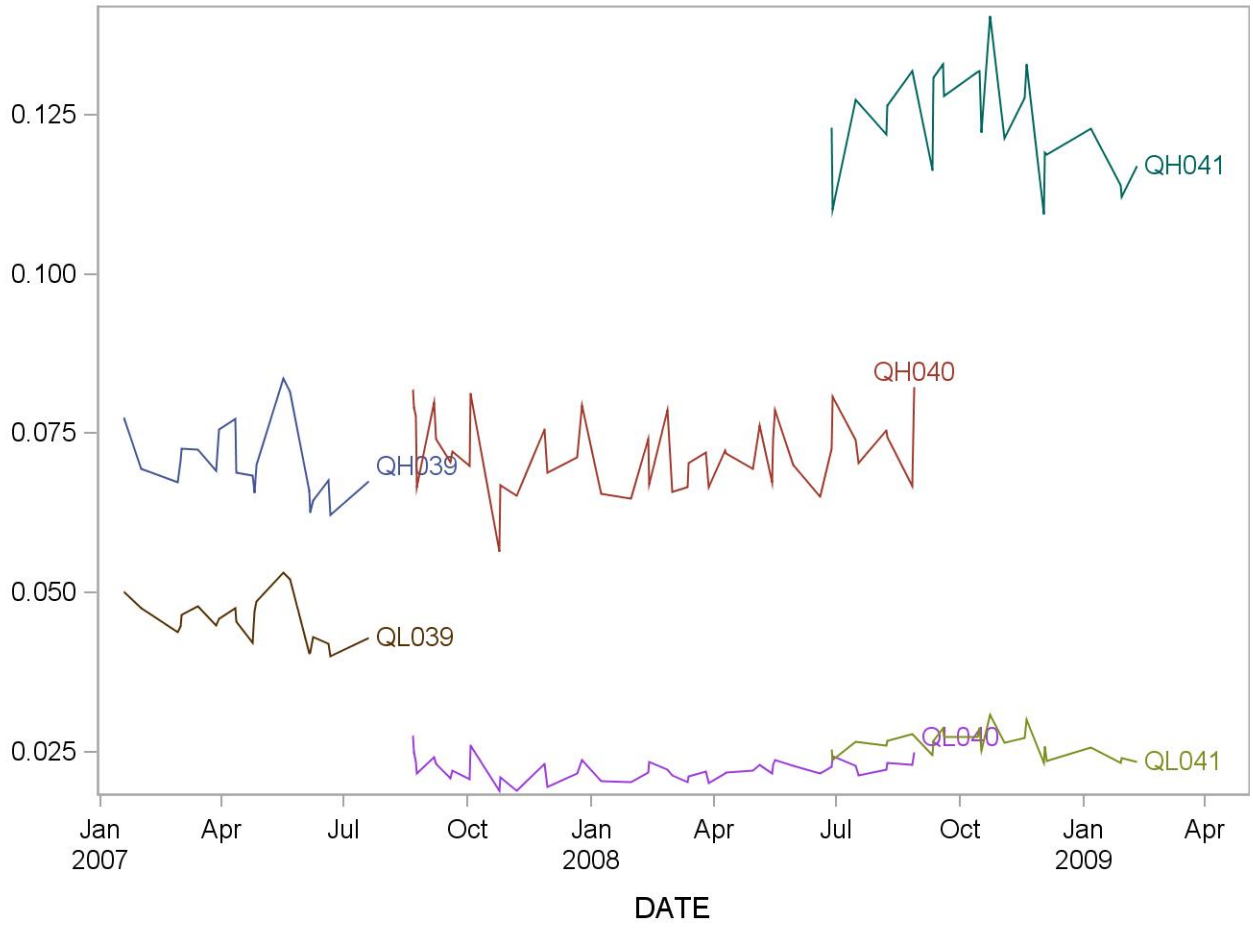
9. Summary Statistics and Graphs

See following pages.

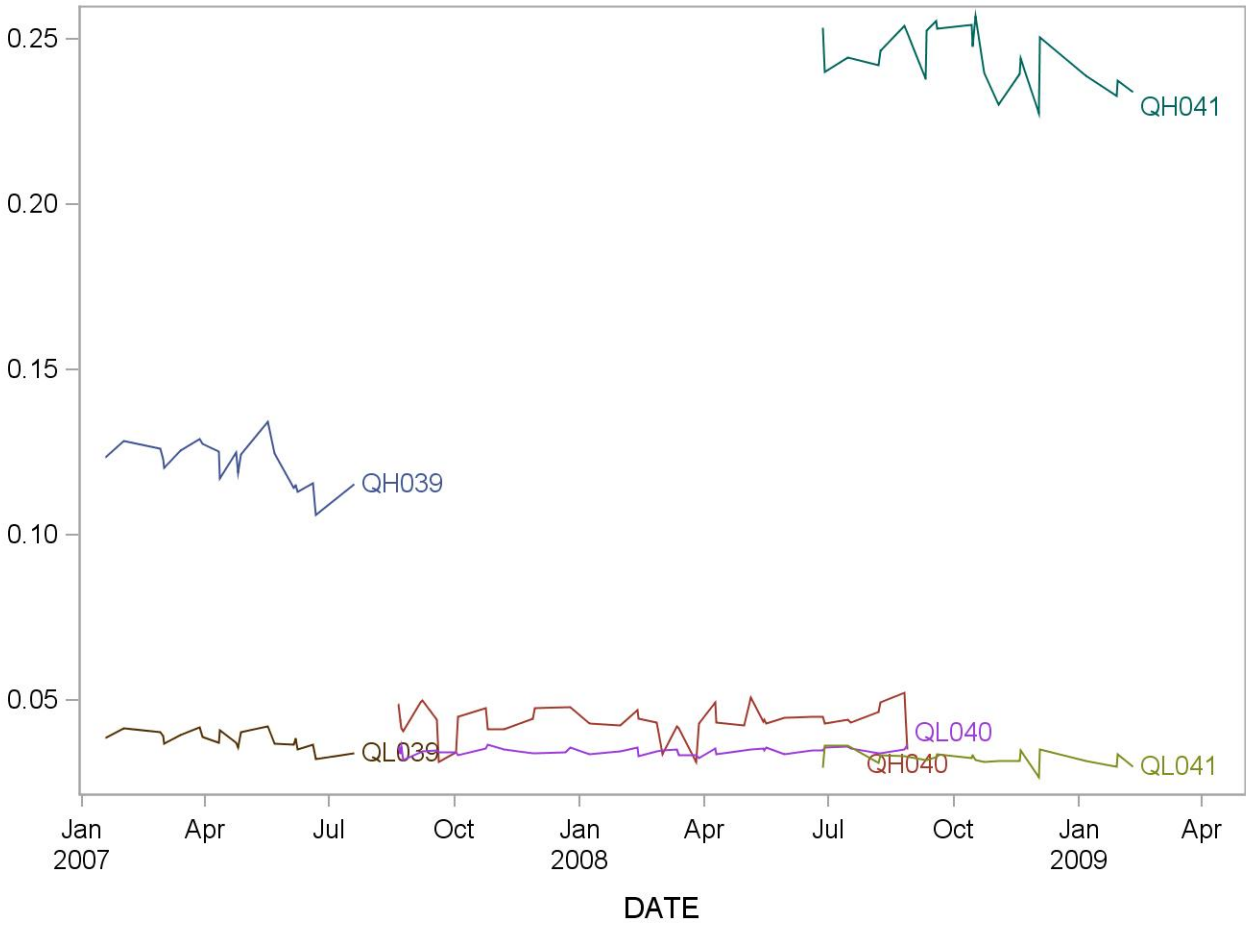
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	29	18JAN07	19JUL07	1.12643	0.09052	8.0
QL039	29	18JAN07	19JUL07	0.27112	0.02235	8.2
QH040	53	21AUG07	28AUG08	0.88964	0.03525	4.0
QL040	53	21AUG07	28AUG08	0.22788	0.00899	3.9
QH041	27	27JUN08	03FEB09	1.15726	0.06344	5.5
QL041	27	27JUN08	03FEB09	0.25377	0.01403	5.5



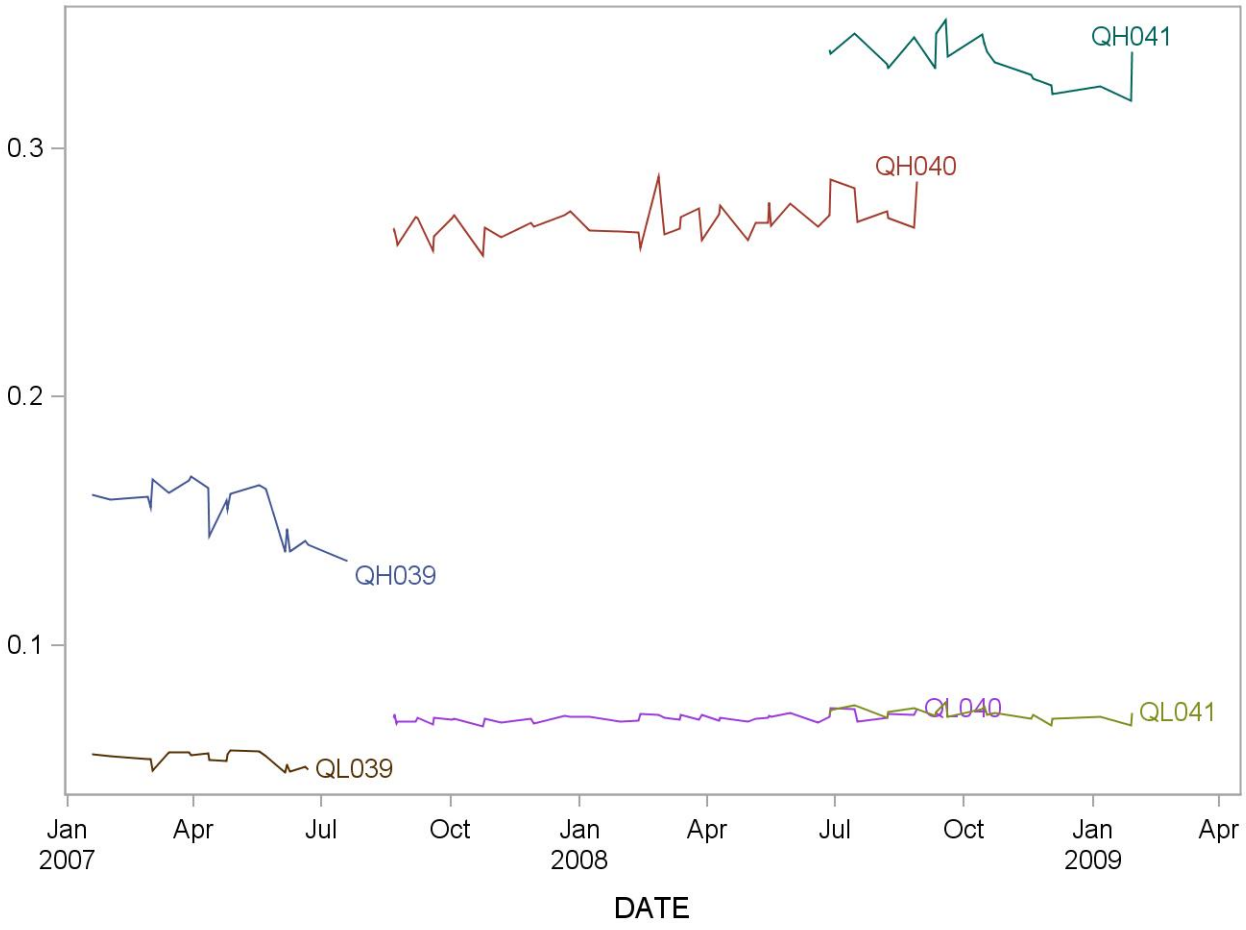
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.07343	0.00506	6.9
QH039	29	18JAN07	19JUL07	0.07013	0.00566	8.1
QL039	4	18JAN07	31JAN07	0.04884	0.00335	6.9
QL039	29	18JAN07	19JUL07	0.04549	0.00385	8.5
QH040	55	21AUG07	28AUG08	0.07200	0.00625	8.7
QL040	55	21AUG07	28AUG08	0.02241	0.00205	9.1
QH041	29	27JUN08	09FEB09	0.12383	0.00778	6.3
QL041	29	27JUN08	09FEB09	0.02627	0.00205	7.8



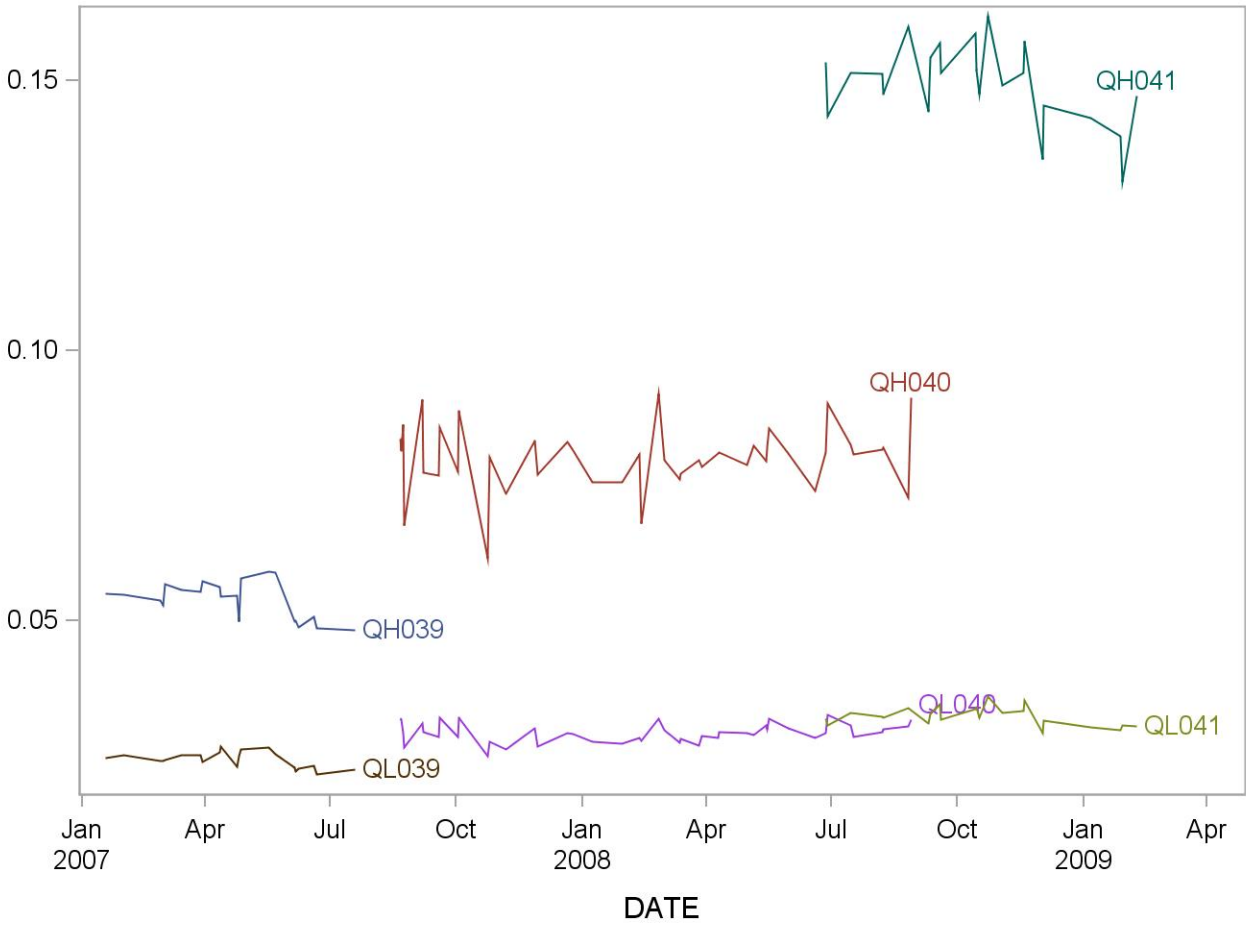
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.12598	0.00310	2.5
QH039	29	18JAN07	19JUL07	0.12154	0.00651	5.4
QL039	4	18JAN07	31JAN07	0.04018	0.00192	4.8
QL039	29	18JAN07	19JUL07	0.03833	0.00255	6.7
QH040	54	21AUG07	28AUG08	0.04381	0.00470	10.7
QL040	55	21AUG07	28AUG08	0.03465	0.00102	2.9
QH041	28	27JUN08	09FEB09	0.24384	0.00885	3.6
QL041	28	27JUN08	09FEB09	0.03240	0.00235	7.3



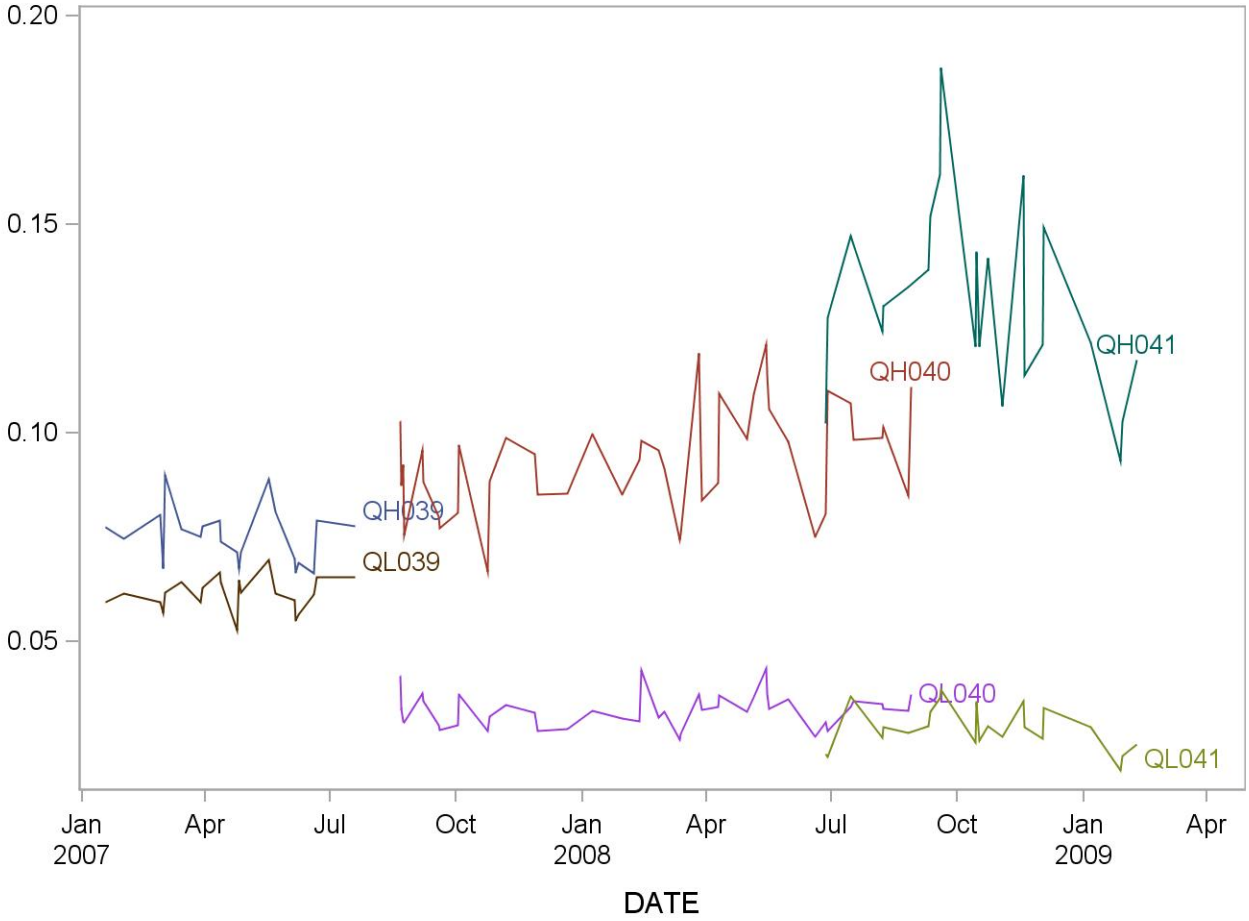
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.15968	0.00660	4.1
QH039	29	18JAN07	19JUL07	0.15419	0.01095	7.1
QL039	4	18JAN07	31JAN07	0.05594	0.00043	0.8
QL039	28	18JAN07	21JUN07	0.05406	0.00307	5.7
QH040	55	21AUG07	28AUG08	0.27034	0.00746	2.8
QL040	55	21AUG07	28AUG08	0.07081	0.00159	2.2
QH041	26	27JUN08	29JAN09	0.33490	0.00914	2.7
QL041	26	27JUN08	29JAN09	0.07259	0.00238	3.3



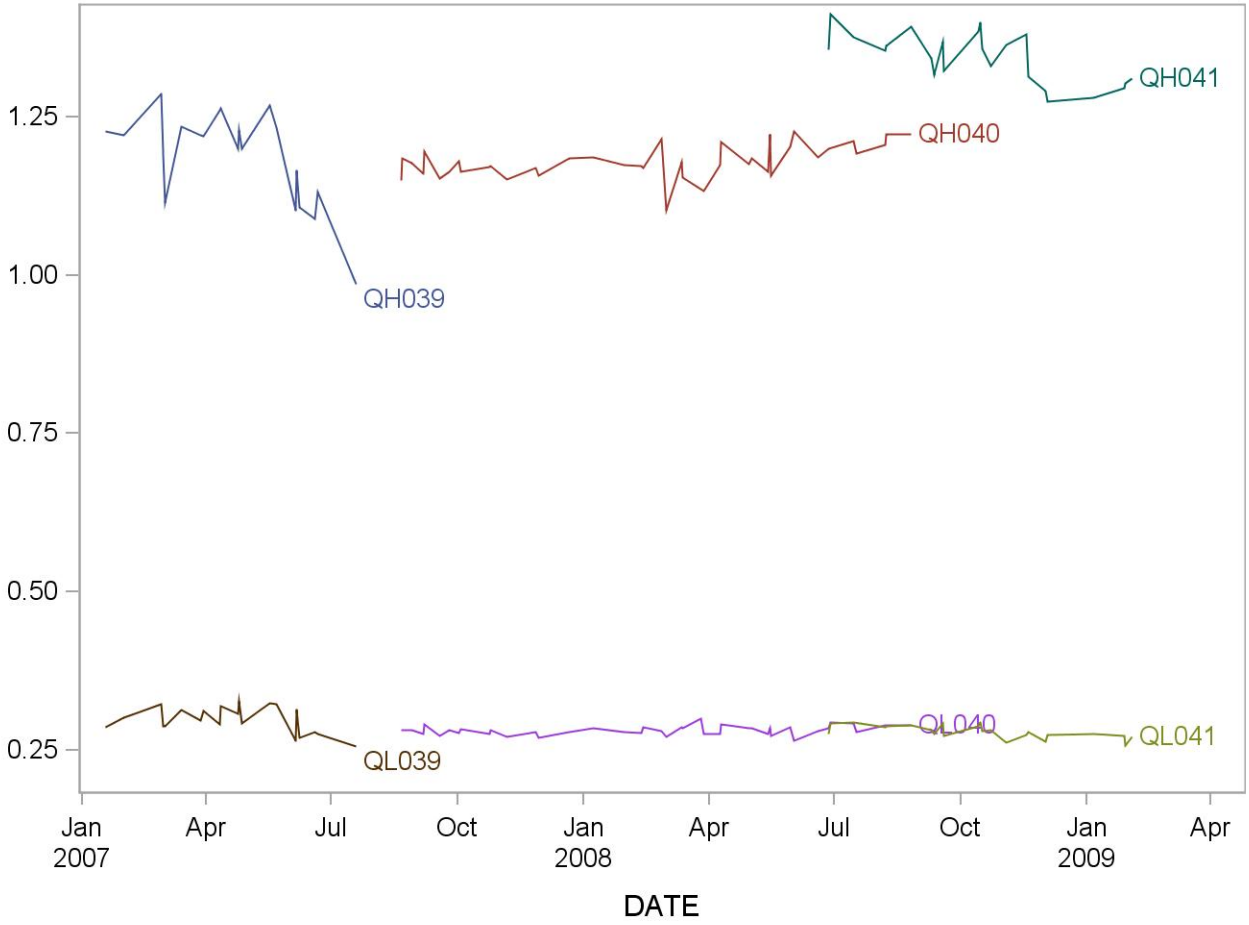
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.05480	0.00054	1.0
QH039	29	18JAN07	19JUL07	0.05346	0.00339	6.3
QL039	4	18JAN07	31JAN07	0.02466	0.00172	7.0
QL039	29	18JAN07	19JUL07	0.02403	0.00155	6.4
QH040	54	21AUG07	28AUG08	0.08010	0.00626	7.8
QL040	53	21AUG07	28AUG08	0.02921	0.00190	6.5
QH041	28	27JUN08	09FEB09	0.14872	0.00740	5.0
QL041	28	27JUN08	09FEB09	0.03203	0.00182	5.7



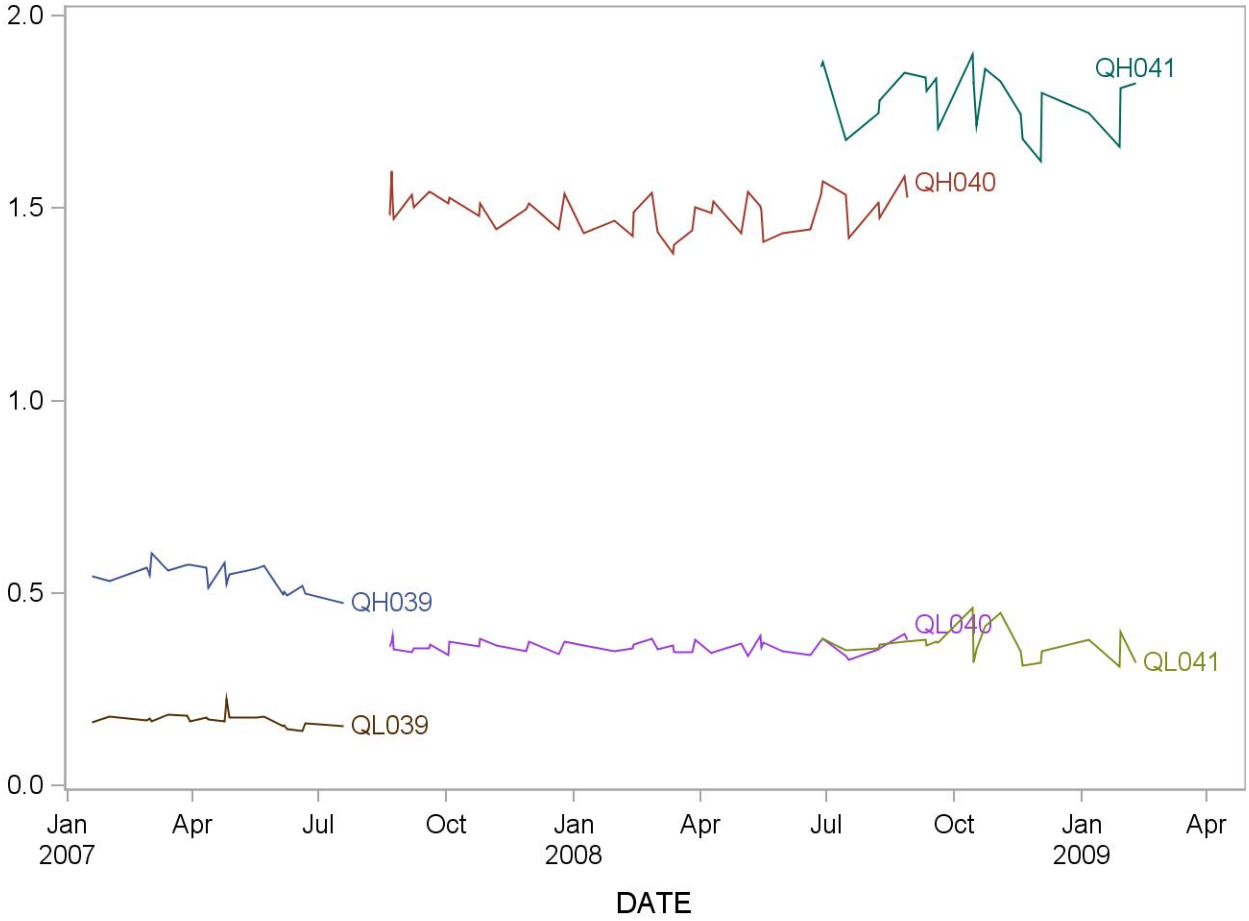
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.07598	0.00324	4.3
QH039	29	18JAN07	19JUL07	0.07488	0.00776	10.4
QL039	4	18JAN07	31JAN07	0.06041	0.00664	11.0
QL039	29	18JAN07	19JUL07	0.06099	0.00619	10.1
QH040	53	21AUG07	28AUG08	0.09336	0.01319	14.1
QL040	53	21AUG07	28AUG08	0.03351	0.00442	13.2
QH041	28	27JUN08	09FEB09	0.13134	0.02185	16.6
QL041	28	27JUN08	09FEB09	0.02939	0.00511	17.4



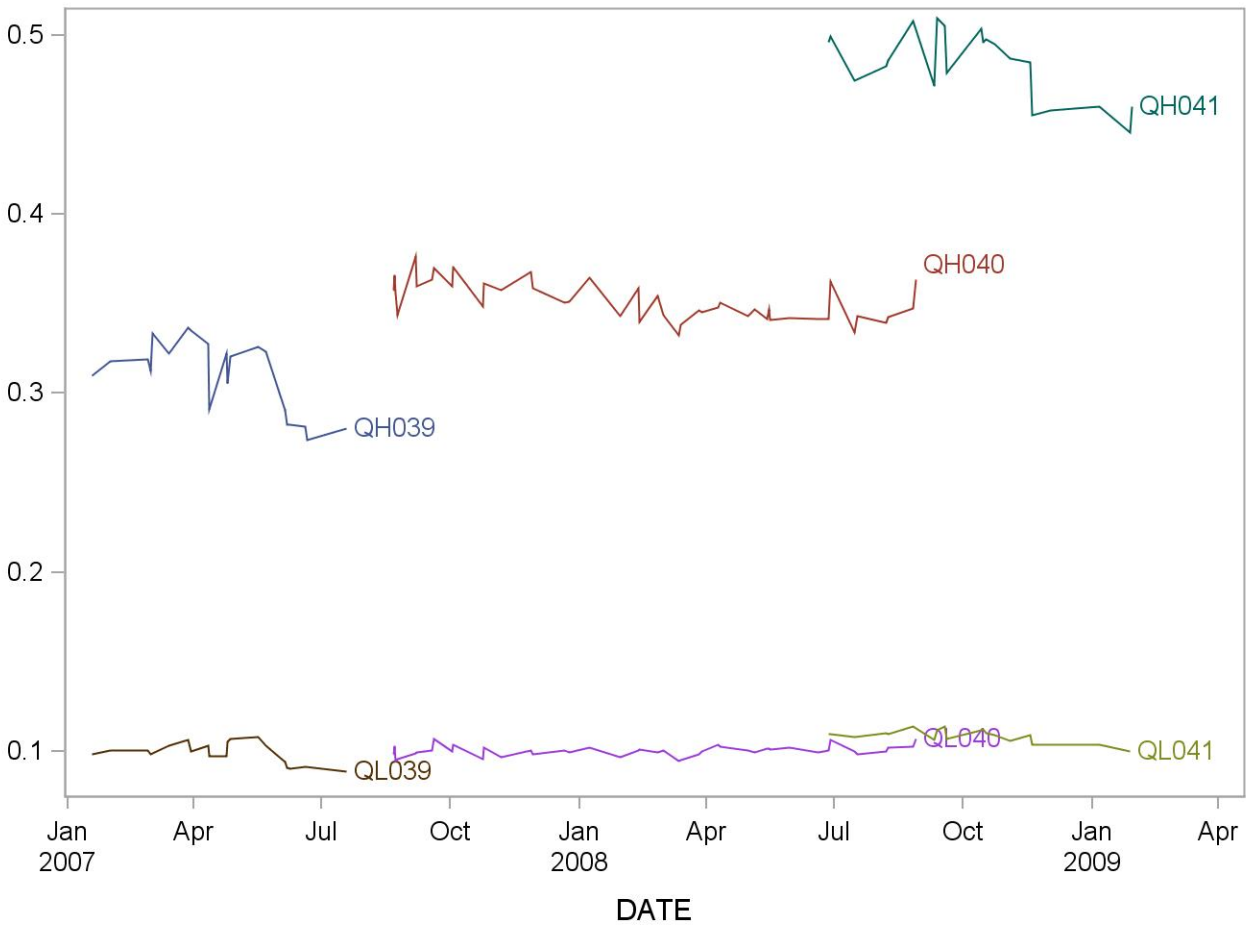
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	29	18JAN07	19JUL07	1.19046	0.07607	6.4
QL039	29	18JAN07	19JUL07	0.29811	0.02176	7.3
QH040	51	21AUG07	26AUG08	1.17762	0.02744	2.3
QL040	54	21AUG07	26AUG08	0.28165	0.00717	2.5
QH041	28	27JUN08	03FEB09	1.34046	0.04214	3.1
QL041	28	27JUN08	03FEB09	0.28023	0.01036	3.7



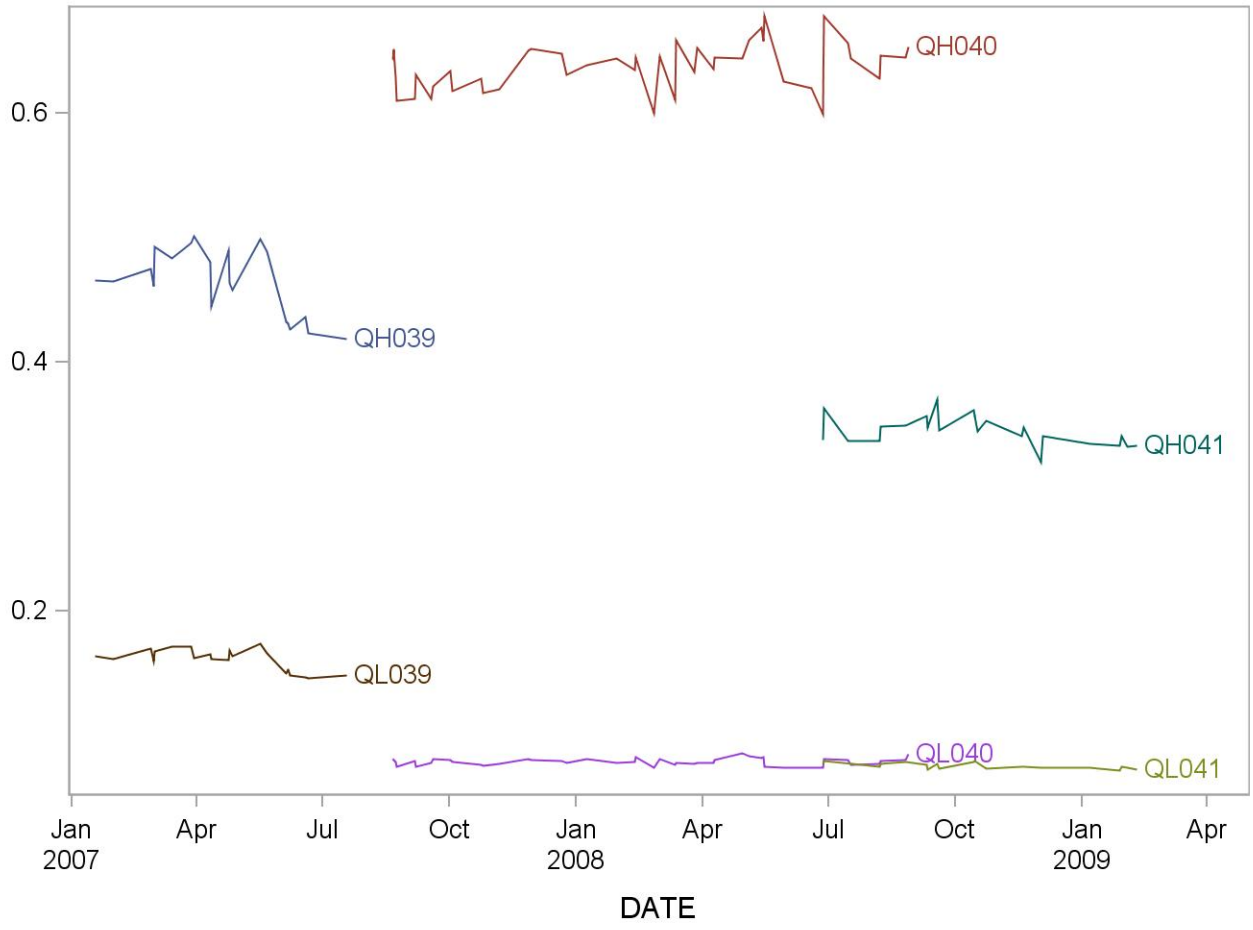
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.53808	0.01799	3.3
QH039	29	18JAN07	19JUL07	0.53861	0.03461	6.4
QL039	4	18JAN07	31JAN07	0.17189	0.01591	9.3
QL039	29	18JAN07	19JUL07	0.17036	0.01715	10.1
QH040	55	21AUG07	28AUG08	1.49047	0.05233	3.5
QL040	55	21AUG07	28AUG08	0.36095	0.01678	4.6
QH041	28	27JUN08	09FEB09	1.78457	0.08297	4.6
QL041	28	27JUN08	09FEB09	0.36708	0.04202	11.4



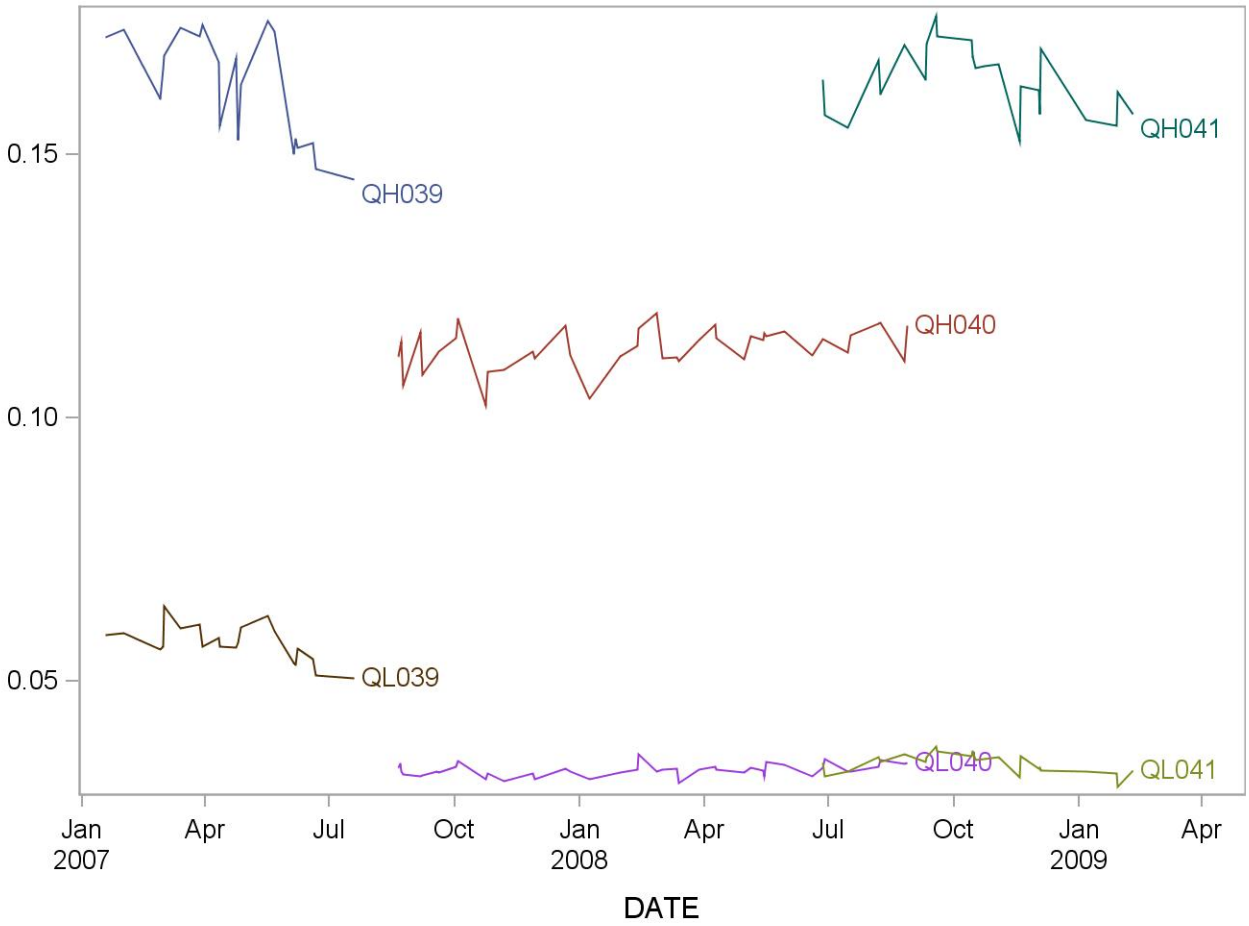
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	29	18JAN07	19JUL07	0.30833	0.01996	6.5
QL039	28	18JAN07	19JUL07	0.09867	0.00577	5.9
QH040	55	21AUG07	28AUG08	0.35198	0.01221	3.5
QL040	55	21AUG07	28AUG08	0.10012	0.00293	2.9
QH041	26	27JUN08	29JAN09	0.48209	0.01916	4.0
QL041	23	27JUN08	28JAN09	0.10853	0.00366	3.4



Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.46494	0.01348	2.9
QH039	29	18JAN07	19JUL07	0.46155	0.02683	5.8
QL039	4	18JAN07	31JAN07	0.16231	0.00317	2.0
QL039	29	18JAN07	19JUL07	0.16064	0.00947	5.9
QH040	55	21AUG07	28AUG08	0.63665	0.01965	3.1
QL040	55	21AUG07	28AUG08	0.07845	0.00321	4.1
QH041	27	27JUN08	09FEB09	0.34504	0.01188	3.4
QL041	26	27JUN08	09FEB09	0.07551	0.00247	3.3



Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.17301	0.00230	1.3
QH039	29	18JAN07	19JUL07	0.16301	0.01045	6.4
QL039	4	18JAN07	31JAN07	0.05884	0.00152	2.6
QL039	29	18JAN07	19JUL07	0.05709	0.00338	5.9
QH040	54	21AUG07	28AUG08	0.11315	0.00495	4.4
QL040	54	21AUG07	28AUG08	0.03298	0.00134	4.1
QH041	29	27JUN08	09FEB09	0.16367	0.00633	3.9
QL041	29	27JUN08	09FEB09	0.03424	0.00183	5.3



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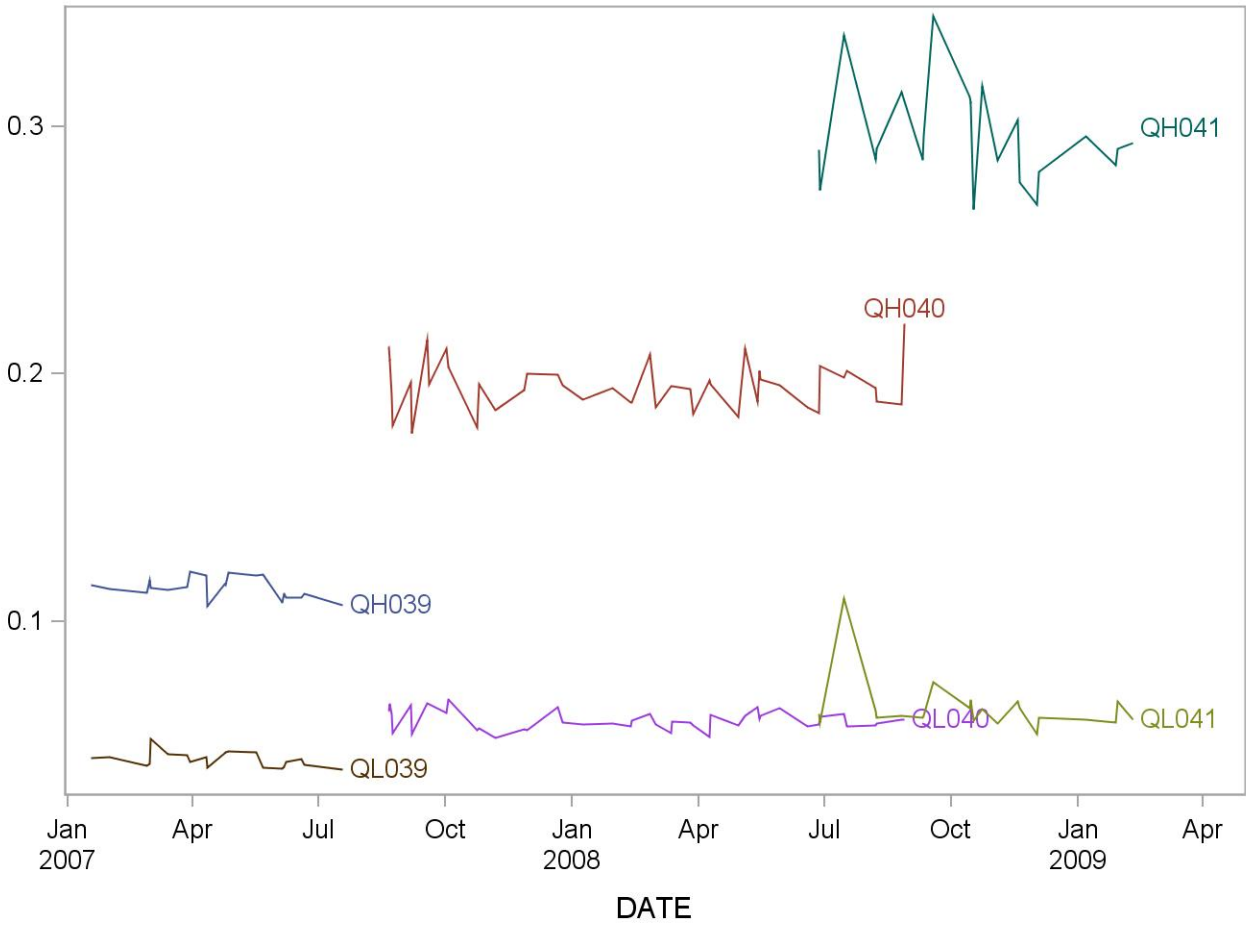
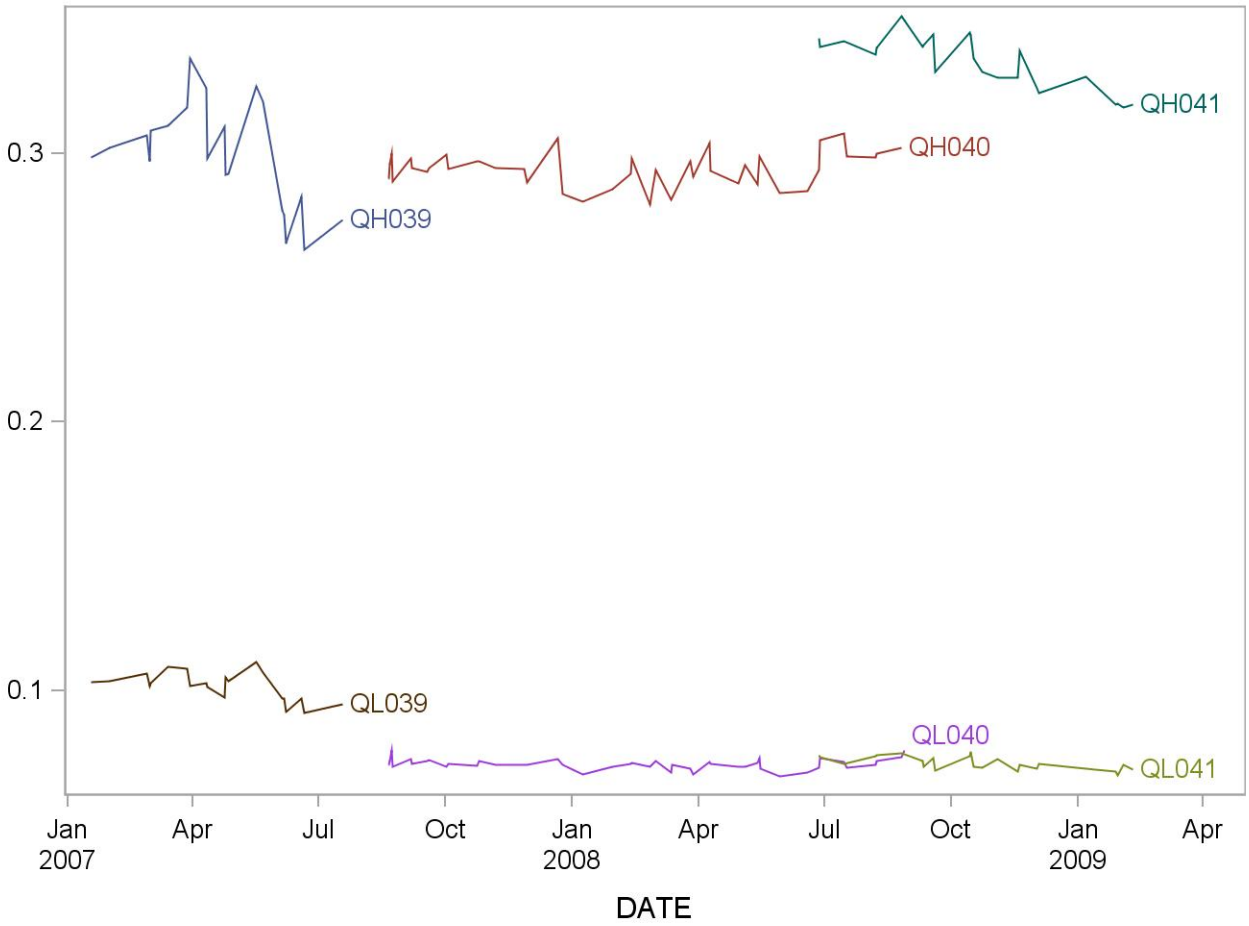
QH039	4	18JAN07	31JAN07	0.11389	0.00090	0.8
QH039	29	18JAN07	19JUL07	0.11304	0.00449	4.0
QL039	4	18JAN07	31JAN07	0.04506	0.00160	3.5
QL039	29	18JAN07	19JUL07	0.04413	0.00311	7.1
QH040	55	21AUG07	28AUG08	0.19525	0.01119	5.7
QL040	55	21AUG07	28AUG08	0.06028	0.00426	7.1
QH041	27	27JUN08	09FEB09	0.29568	0.01856	6.3
QL041	27	27JUN08	09FEB09	0.06447	0.00988	15.3



Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QH039	4	18JAN07	31JAN07	0.30031	0.00821	2.7
QH039	29	18JAN07	19JUL07	0.29781	0.01937	6.5
QL039	4	18JAN07	31JAN07	0.10329	0.00127	1.2
QL039	29	18JAN07	19JUL07	0.10167	0.00568	5.6
QH040	51	21AUG07	26AUG08	0.29375	0.00691	2.4
QL040	55	21AUG07	28AUG08	0.07233	0.00213	2.9
QH041	28	27JUN08	09FEB09	0.33426	0.00990	3.0
QL041	28	27JUN08	09FEB09	0.07308	0.00254	3.5



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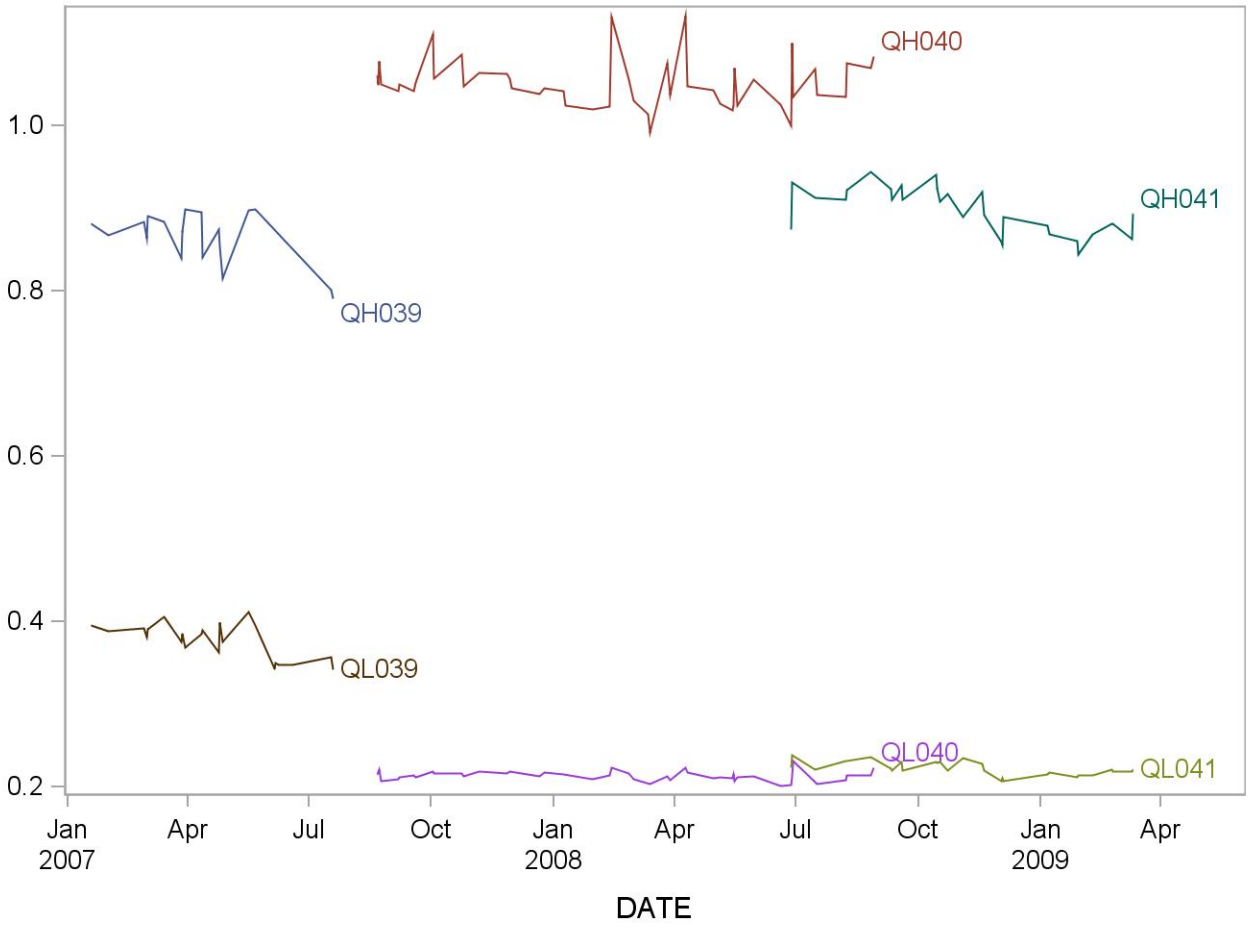
QH039	4	18JAN07	31JAN07	0.87481	0.02310	2.6
QH039	26	18JAN07	19JUL07	0.86630	0.03403	3.9
QL039	4	18JAN07	31JAN07	0.39226	0.00710	1.8
QL039	32	18JAN07	19JUL07	0.37851	0.02242	5.9
QH040	57	21AUG07	28AUG08	1.05088	0.02966	2.8
QL040	55	21AUG07	28AUG08	0.21366	0.00652	3.1
QH041	37	27JUN08	11MAR09	0.89859	0.02926	3.3
QL041	37	27JUN08	11MAR09	0.22217	0.00827	3.7



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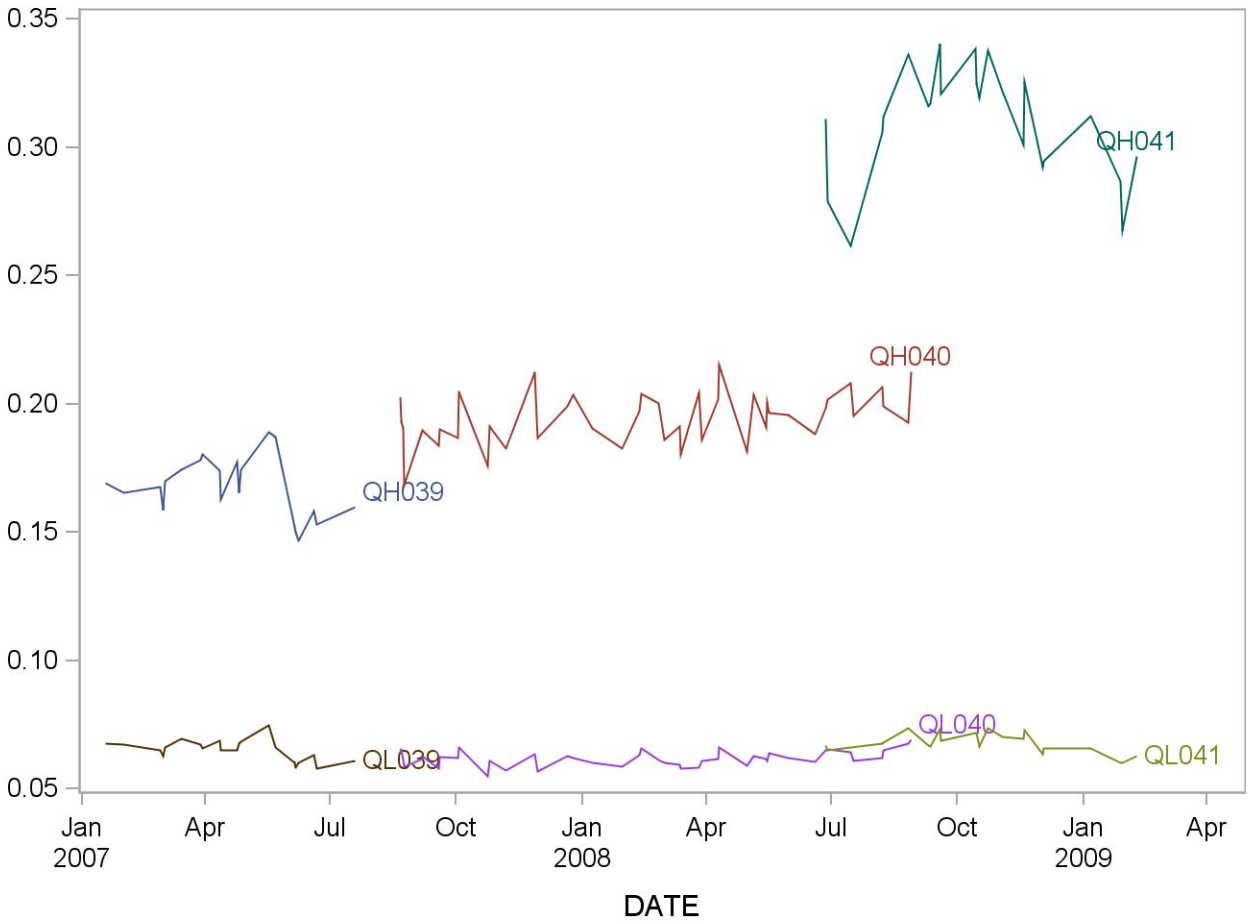
QH039	4	18JAN07	31JAN07	0.16721	0.00386	2.3
QH039	29	18JAN07	19JUL07	0.16594	0.01206	7.3
QL039	4	18JAN07	31JAN07	0.06751	0.00211	3.1
QL039	28	18JAN07	19JUL07	0.06517	0.00426	6.5
QH040	55	21AUG07	28AUG08	0.19458	0.01097	5.6
QL040	55	21AUG07	28AUG08	0.06185	0.00323	5.2
QH041	28	27JUN08	09FEB09	0.31049	0.02087	6.7
QL041	27	27JUN08	09FEB09	0.06773	0.00390	5.8



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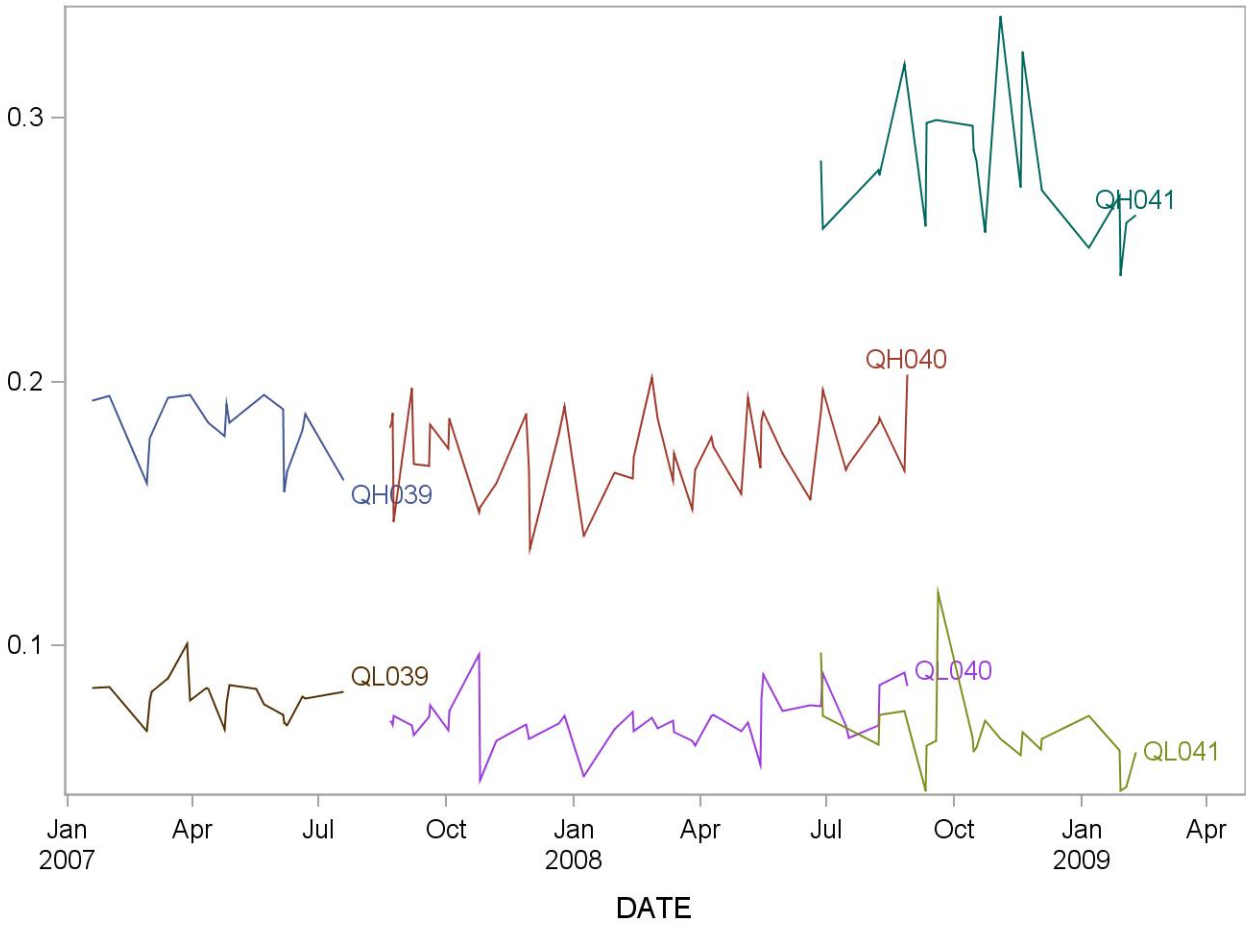
QH039	22	18JAN07	19JUL07	0.18377	0.01306	7.1
QL039	3	18JAN07	31JAN07	0.08423	0.00398	4.7
QL039	28	18JAN07	19JUL07	0.07942	0.00845	10.6
QH040	55	21AUG07	28AUG08	0.17382	0.01714	9.9
QL040	55	21AUG07	28AUG08	0.07149	0.00968	13.5
QH041	26	27JUN08	09FEB09	0.27905	0.02532	9.1
QL041	27	27JUN08	09FEB09	0.06618	0.01682	25.4



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QH039	28	18JAN07	19JUL07	0.02934	0.00328	11.2
QL039	4	18JAN07	31JAN07	0.02270	0.00241	10.6
QL039	29	18JAN07	19JUL07	0.02067	0.00271	13.1
QH040	51	21AUG07	28AUG08	0.12465	0.01935	15.5
QL040	54	21AUG07	28AUG08	0.09553	0.01831	19.2
QL041	26	27JUN08	09FEB09	0.04265	0.00774	18.1
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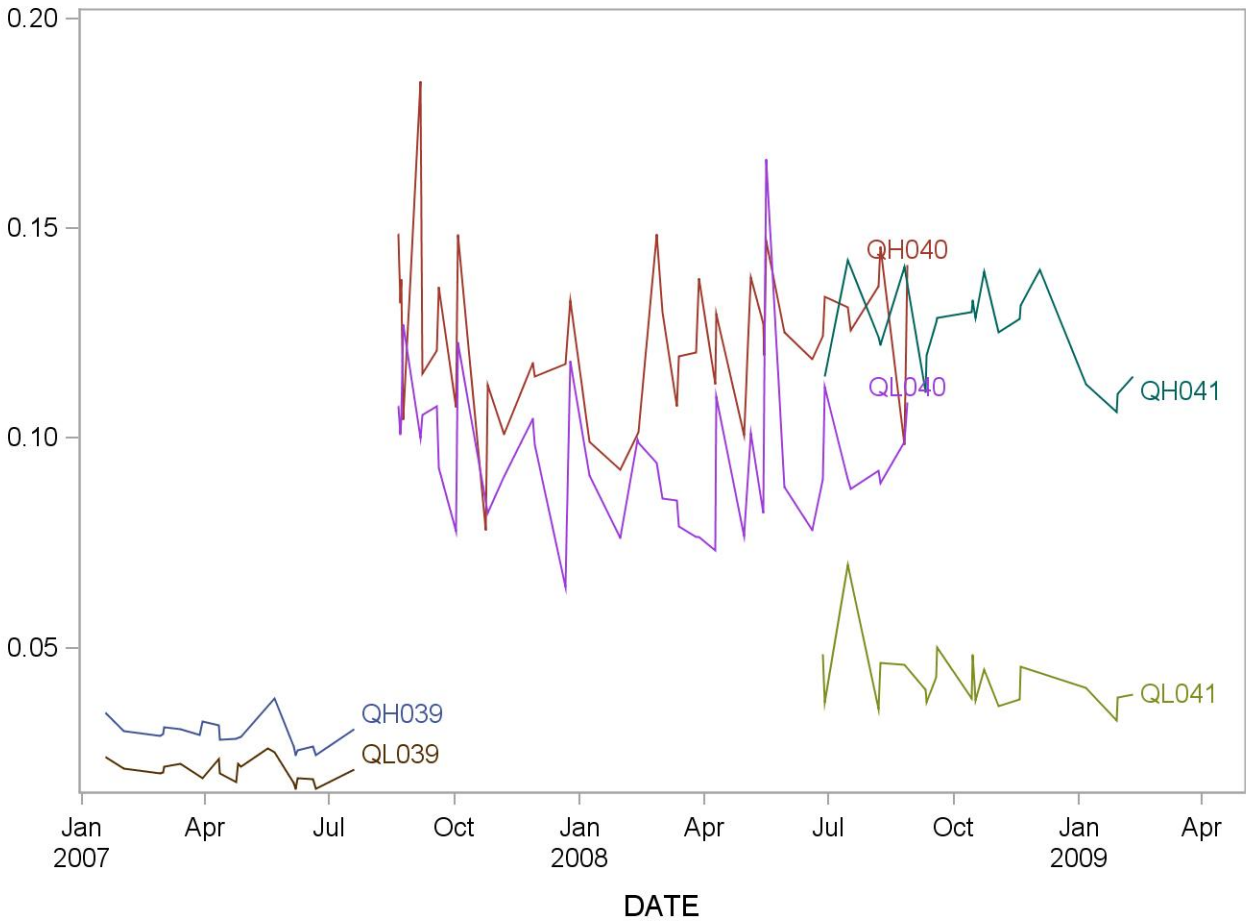

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QL039	3	18JAN07	31JAN07	0.05560	0.00138	2.5
QL039	29	18JAN07	19JUL07	0.05372	0.00284	5.3
QH040	55	21AUG07	28AUG08	0.23415	0.00713	3.0
QL040	55	21AUG07	28AUG08	0.03480	0.00113	3.2
QH041	28	27JUN08	09FEB09	0.16454	0.00500	3.0
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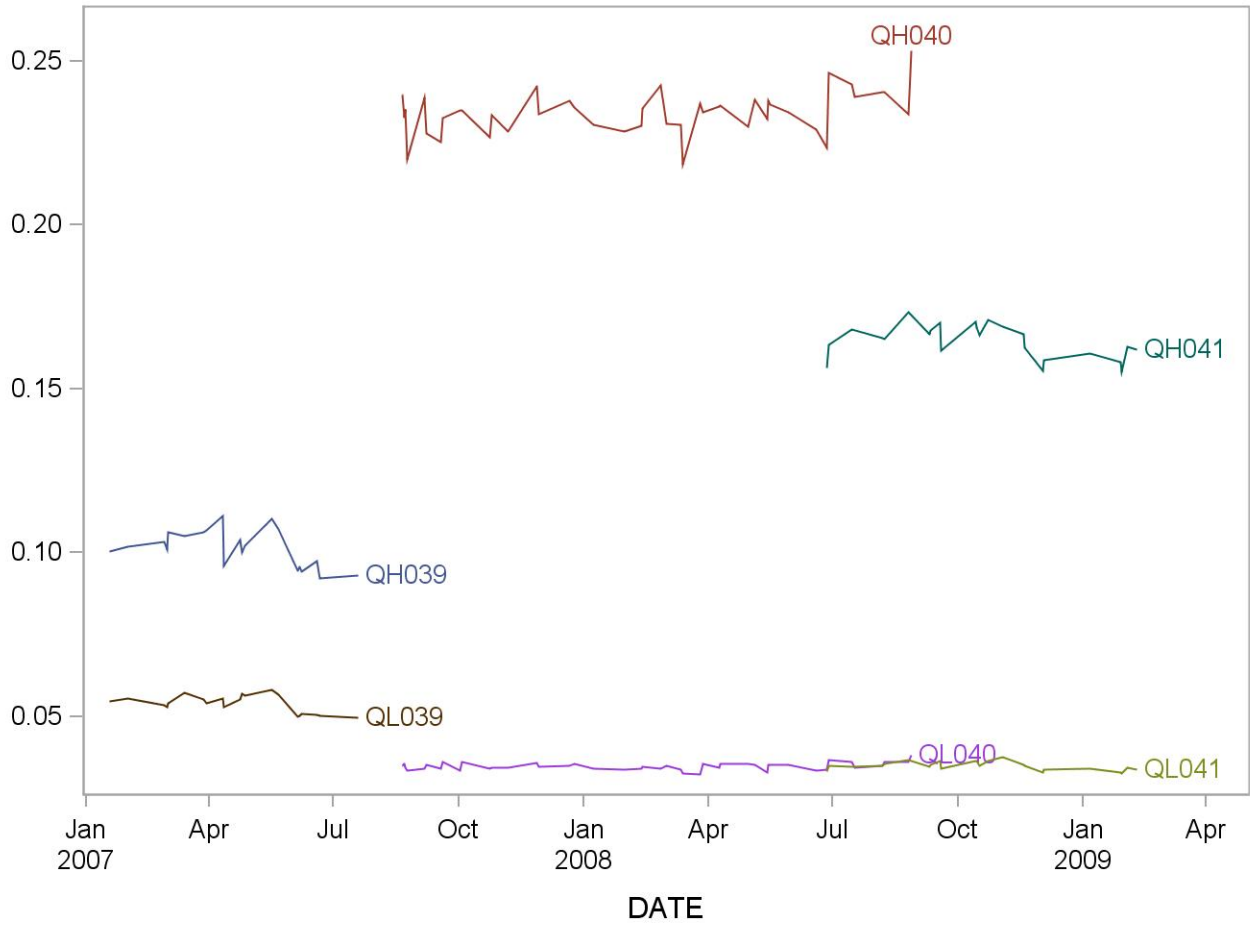

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QH039	29	18JAN07	19JUL07	0.12327	0.00984	8.0
QL039	4	18JAN07	31JAN07	0.05066	0.00293	5.8
QL039	29	18JAN07	19JUL07	0.04925	0.00333	6.8
QH040	54	21AUG07	28AUG08	0.17373	0.01206	6.9
QL040	53	21AUG07	28AUG08	0.05815	0.00359	6.2
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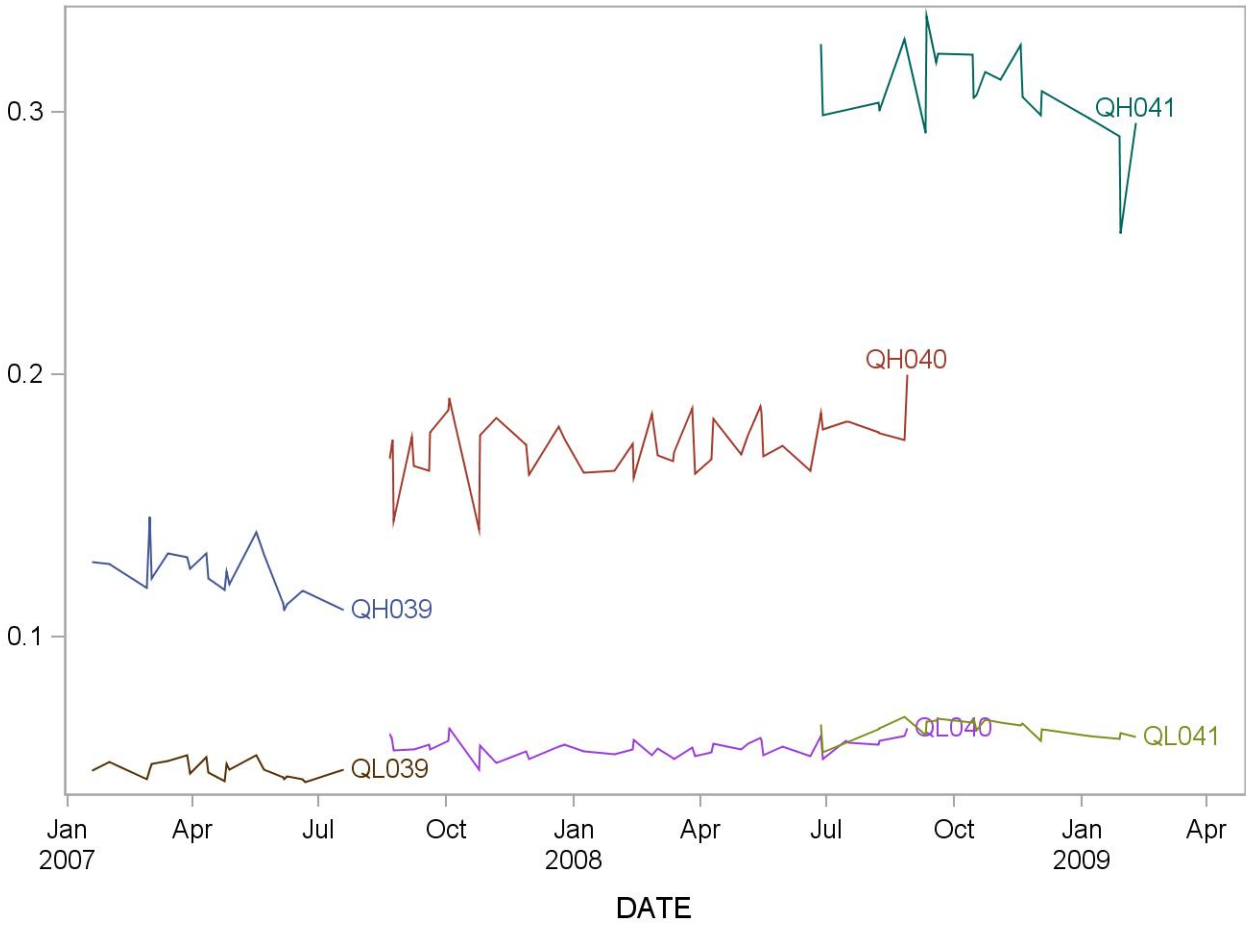
QL041	27	27JUN08	09FEB09	0.06504	0.00334	5.1



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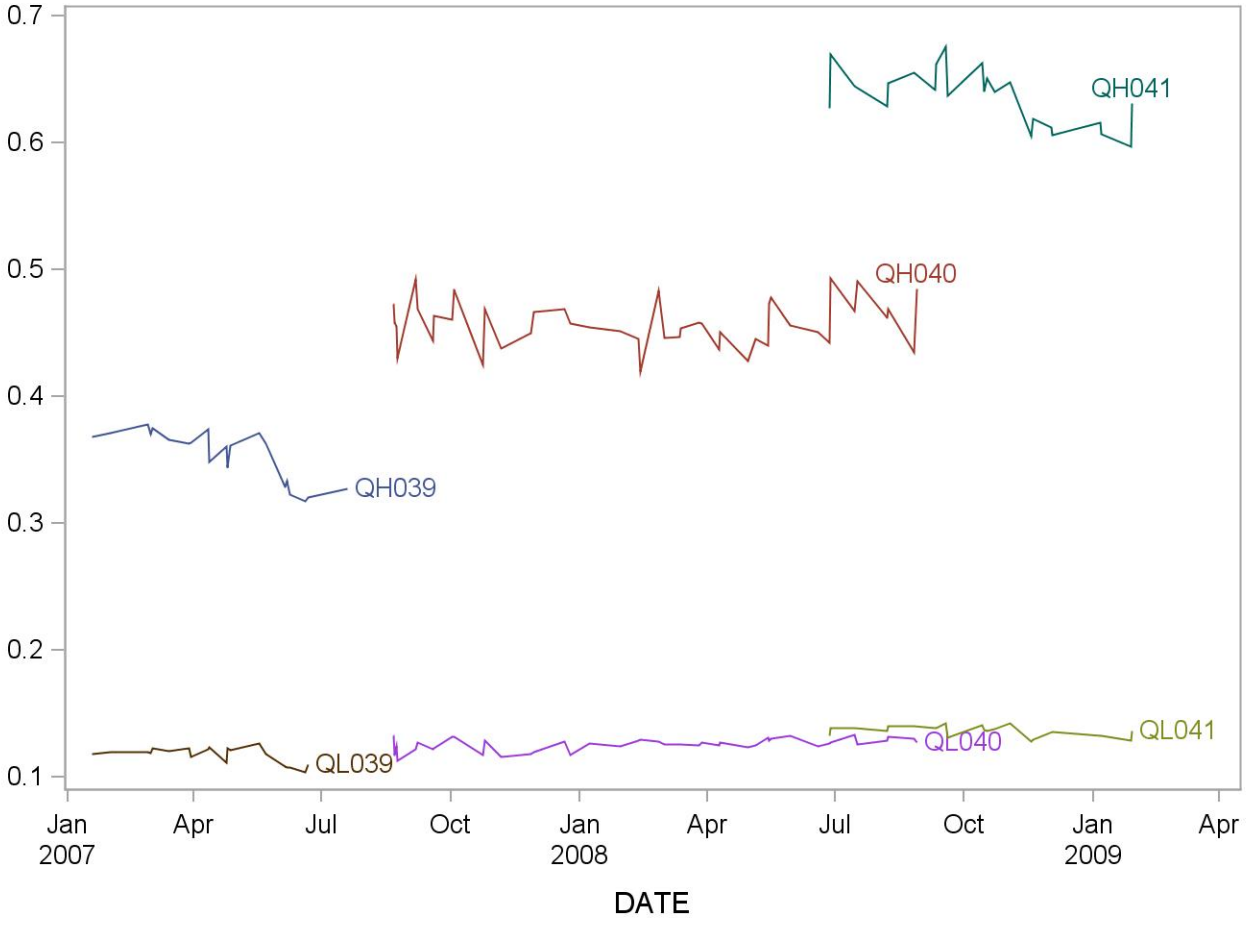
QL039	4	18JAN07	31JAN07	0.11915	0.00412	3.5
QL039	28	18JAN07	21JUN07	0.11669	0.00683	5.9
QH040	55	21AUG07	28AUG08	0.45743	0.01951	4.3
QL040	55	21AUG07	28AUG08	0.12583	0.00570	4.5
QH041	28	27JUN08	29JAN09	0.63528	0.02086	3.3
QL041	27	27JUN08	29JAN09	0.13636	0.00445	3.3



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QH039	24	18JAN07	19JUL07	0.24551	0.01554	6.3
QL039	4	18JAN07	31JAN07	0.38811	0.01200	3.1
QL039	28	18JAN07	19JUL07	0.36891	0.02654	7.2
QH040	52	21AUG07	28AUG08	0.35976	0.02382	6.6
QL040	55	21AUG07	28AUG08	0.18918	0.01708	9.0
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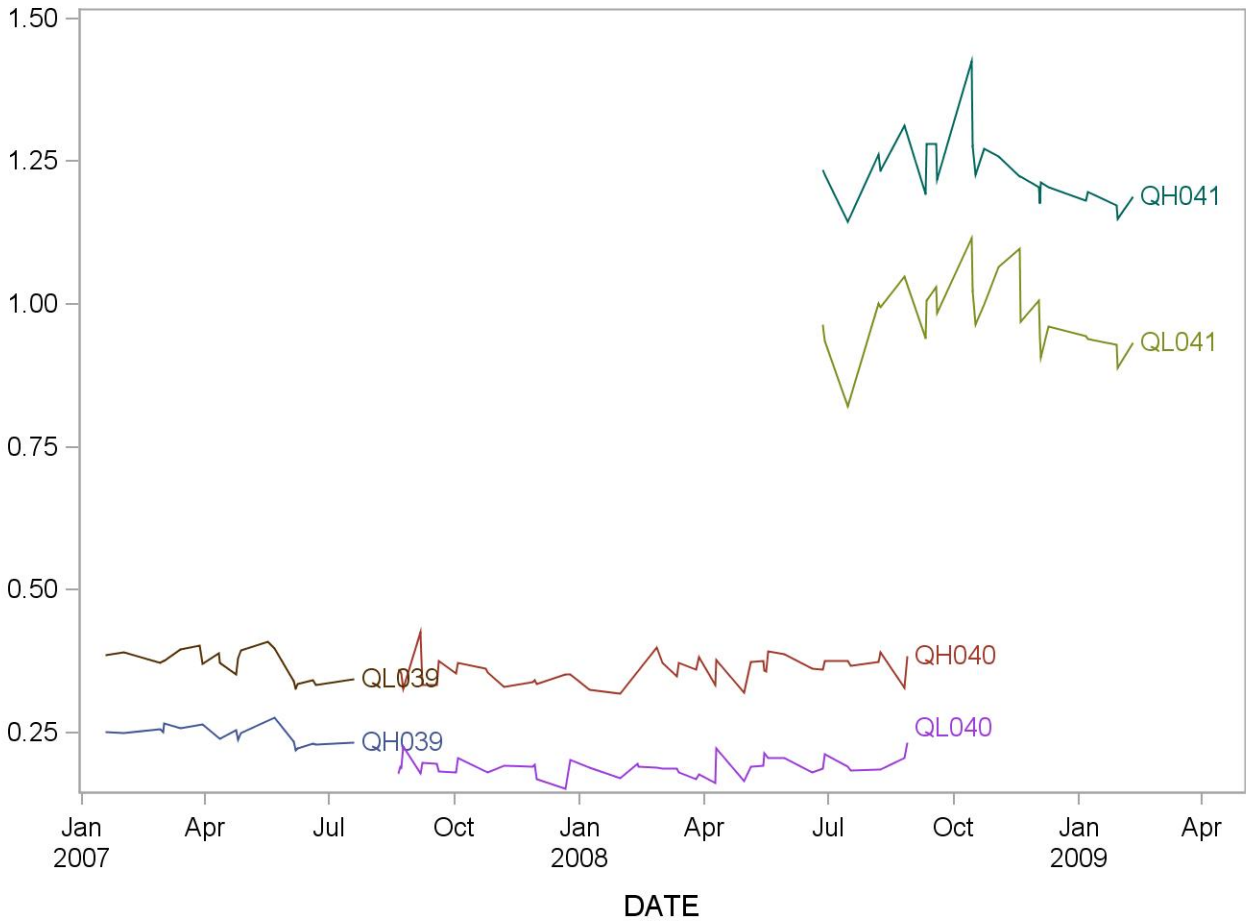
QL041	31	27JUN08	09FEB09	0.97649	0.06096	6.2



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QH040	55	21AUG07	28AUG08	0.1238	0.0130	10.5
QL040	54	21AUG07	28AUG08	0.0489	0.0071	14.6
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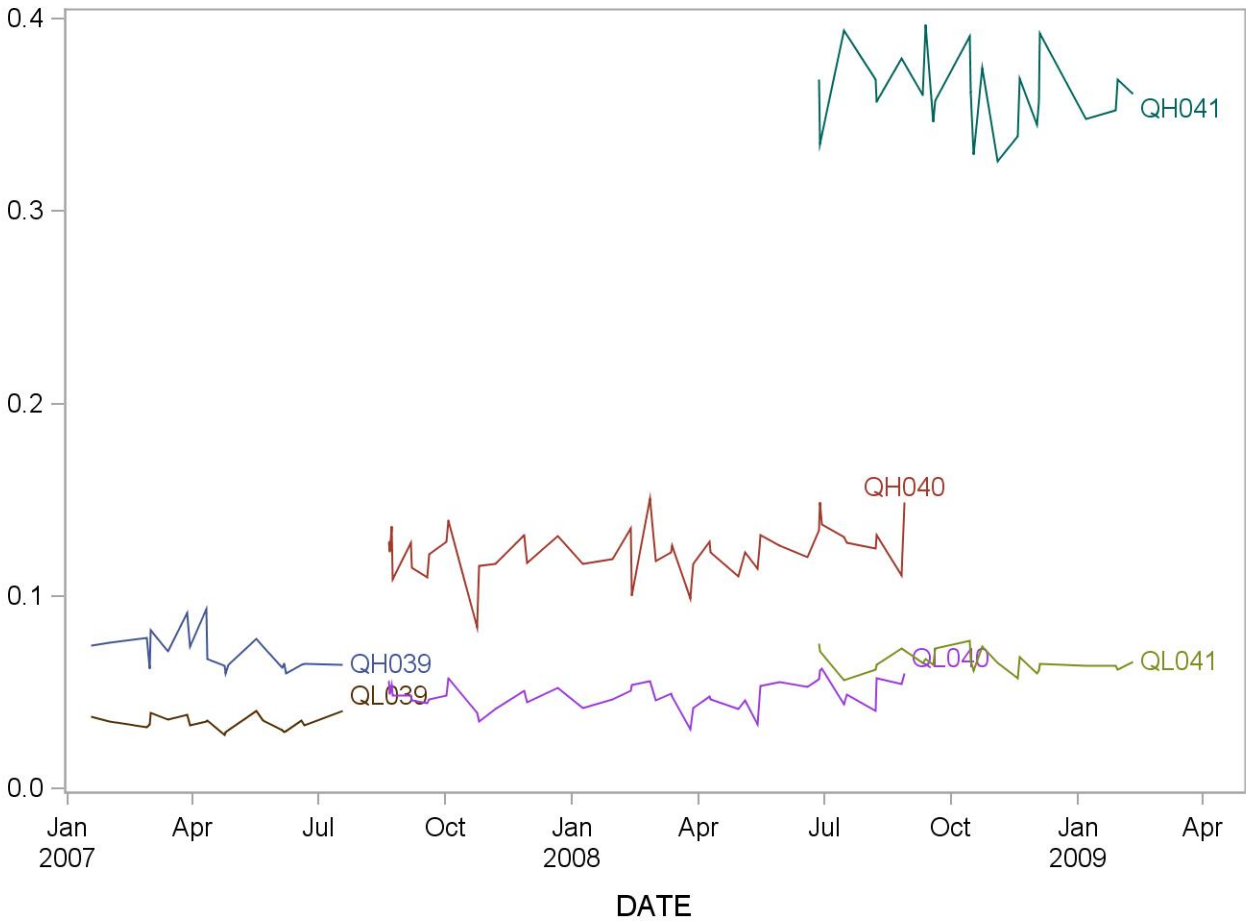

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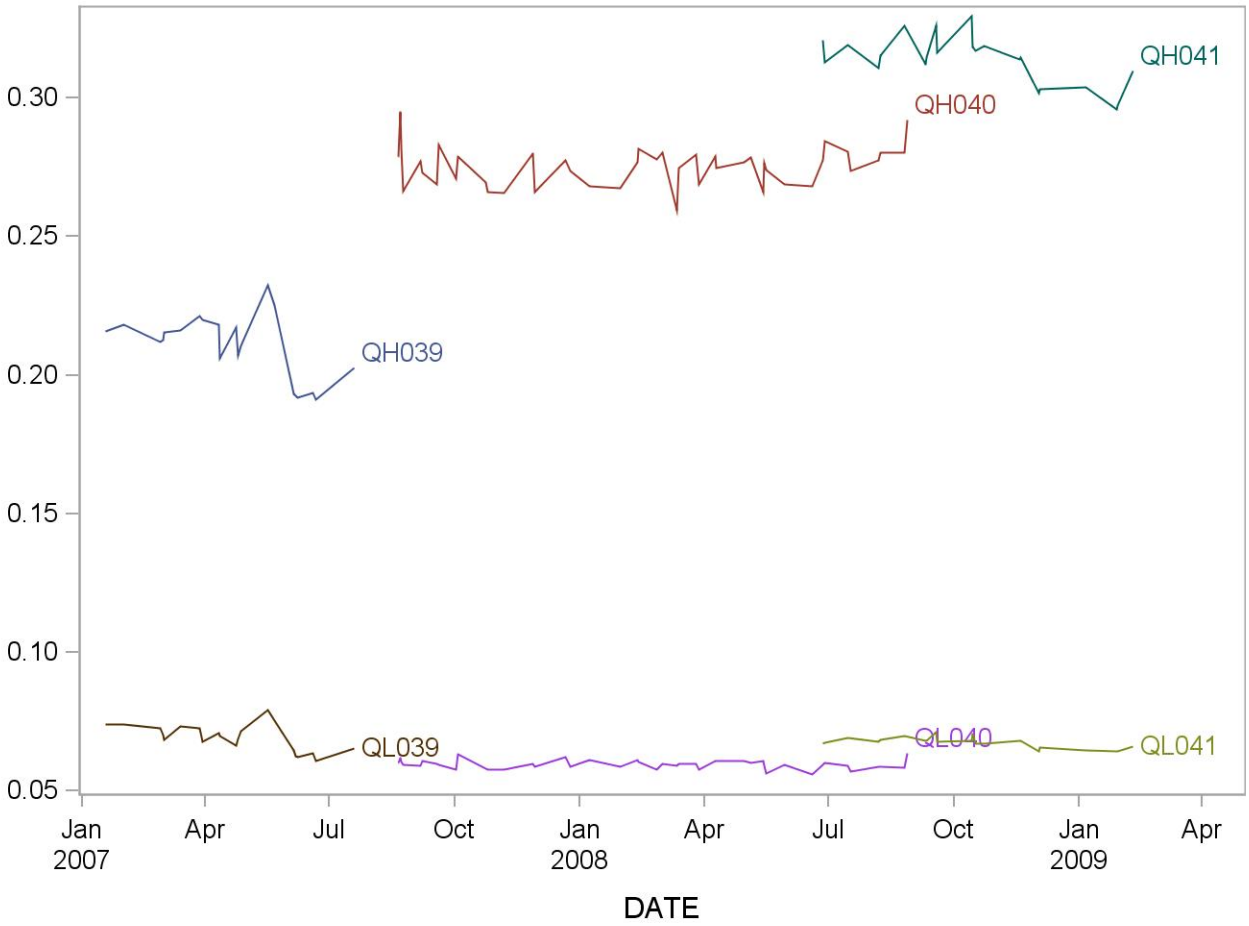
QL039	3	18JAN07	31JAN07	0.07388	0.00136	1.8
QL039	29	18JAN07	19JUL07	0.06930	0.00510	7.4
QH040	55	21AUG07	28AUG08	0.27512	0.00793	2.9
QL040	55	21AUG07	28AUG08	0.05952	0.00191	3.2
QH041	27	27JUN08	09FEB09	0.31297	0.00871	2.8
QL041	27	27JUN08	09FEB09	0.06737	0.00207	3.1



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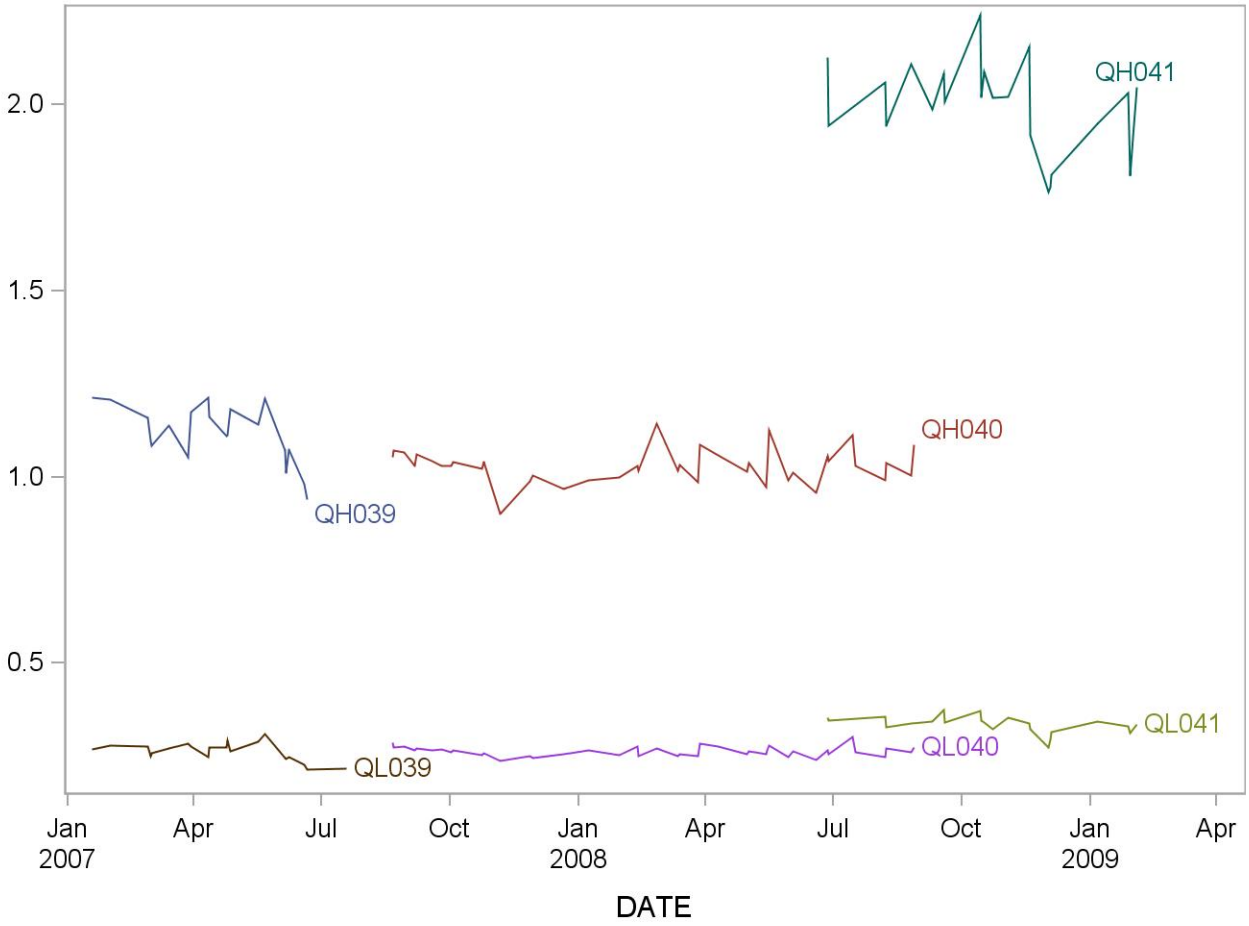
QH039	28	18JAN07	21JUN07	1.11930	0.07742	6.9
QL039	29	18JAN07	19JUL07	0.26309	0.02262	8.6
QH040	53	21AUG07	28AUG08	1.02397	0.05624	5.5
QL040	53	21AUG07	28AUG08	0.26226	0.01453	5.5
QH041	26	27JUN08	03FEB09	1.99036	0.12734	6.4
QL041	26	27JUN08	03FEB09	0.33707	0.02466	7.3



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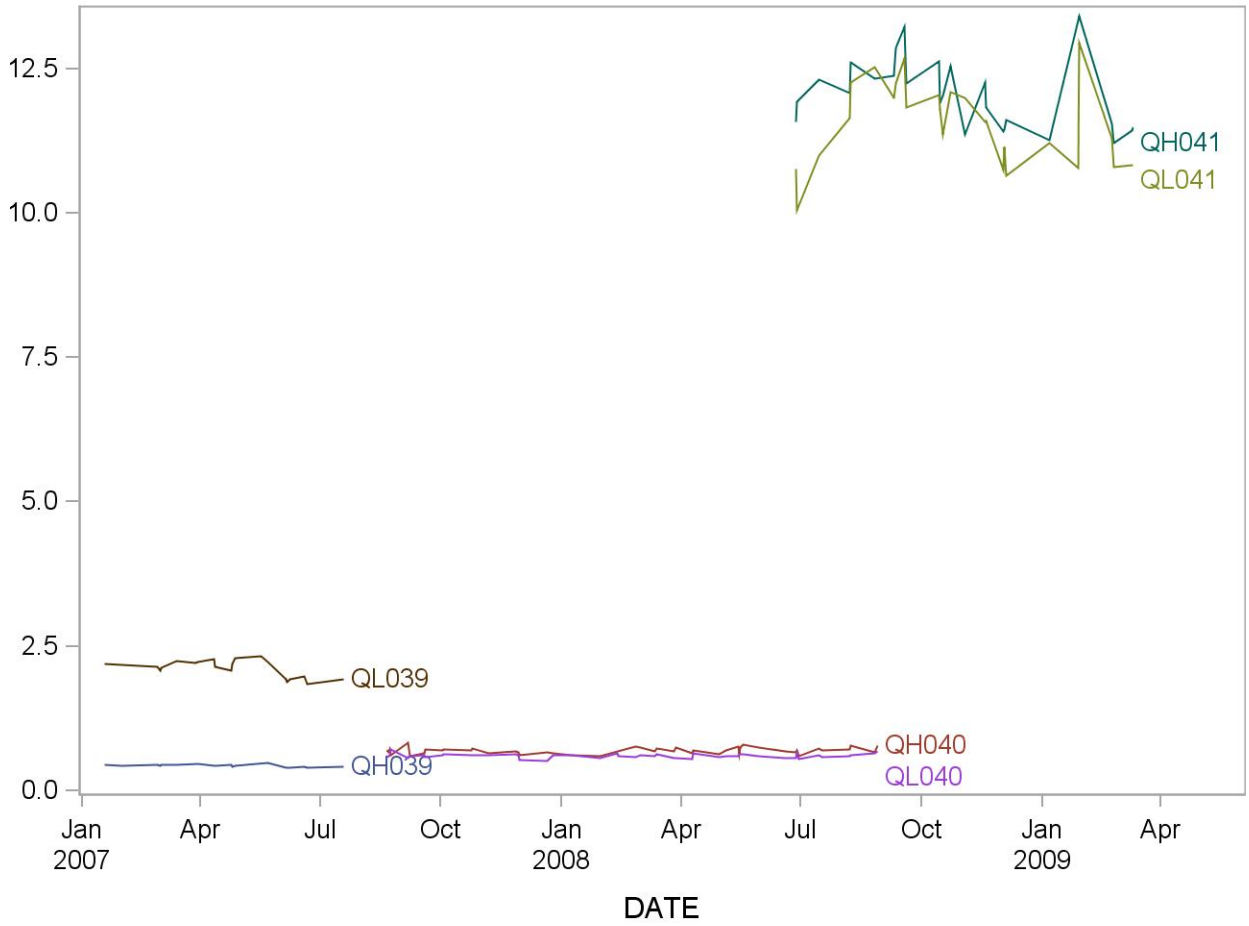
QL039	4	18JAN07	31JAN07	2.18949	0.04315	2.0
QL039	28	18JAN07	19JUL07	2.10786	0.15051	7.1
QH040	53	21AUG07	28AUG08	0.69664	0.05646	8.1
QL040	56	21AUG07	28AUG08	0.60306	0.04077	6.8
QH041	32	27JUN08	11MAR09	11.99113	0.60790	5.1
QL041	33	27JUN08	11MAR09	11.50128	0.70068	6.1



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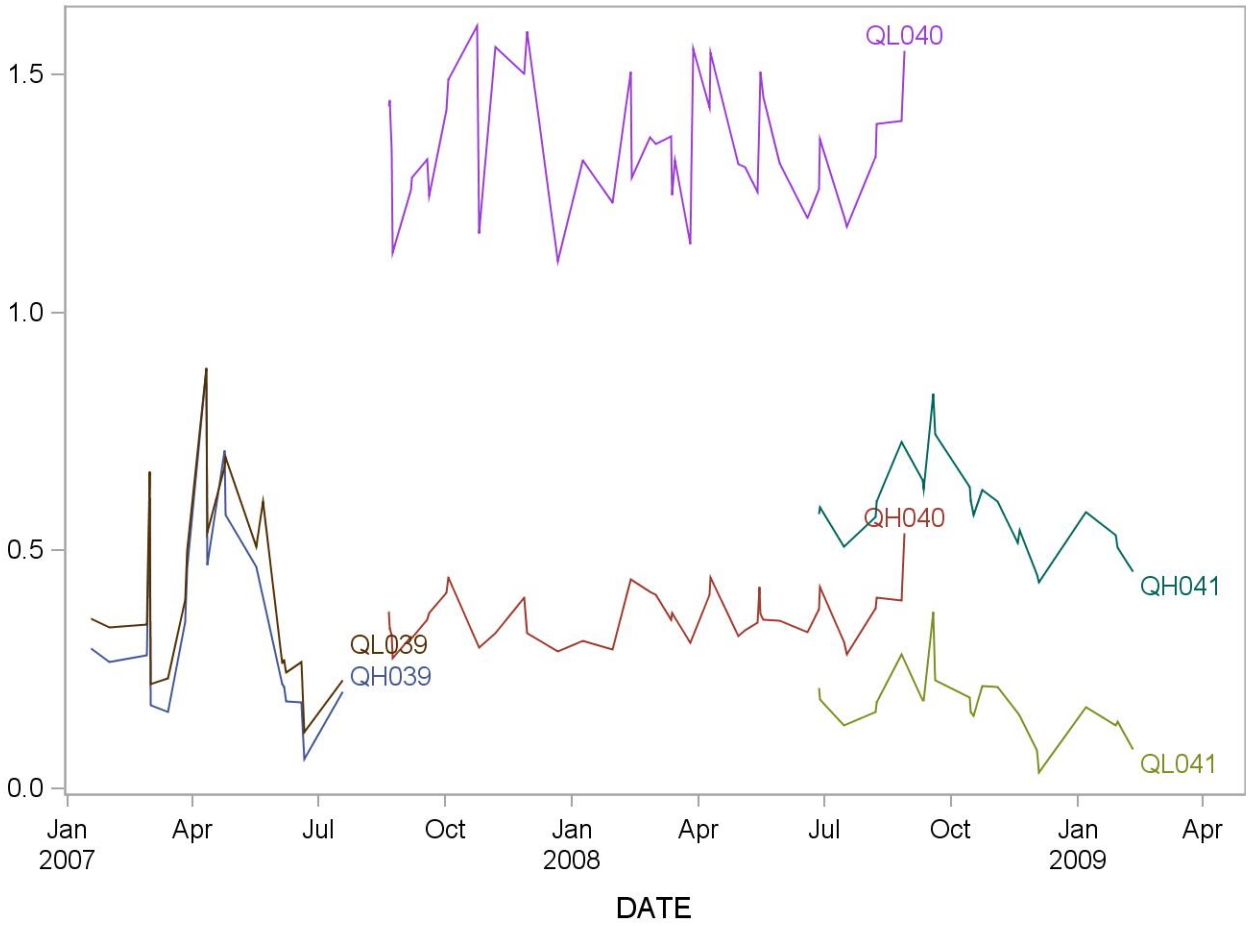
QH039	4	18JAN07	31JAN07	0.28133	0.01935	6.9
QH039	26	18JAN07	19JUL07	0.32328	0.19549	60.5
QL039	4	18JAN07	31JAN07	0.34789	0.02463	7.1
QL039	28	18JAN07	19JUL07	0.38036	0.18553	48.8
QH040	52	21AUG07	28AUG08	0.36024	0.05888	16.3
QL040	55	21AUG07	28AUG08	1.35473	0.13840	10.2
QH041	28	27JUN08	09FEB09	0.58987	0.08909	15.1
QL041	28	27JUN08	09FEB09	0.17482	0.06280	35.9



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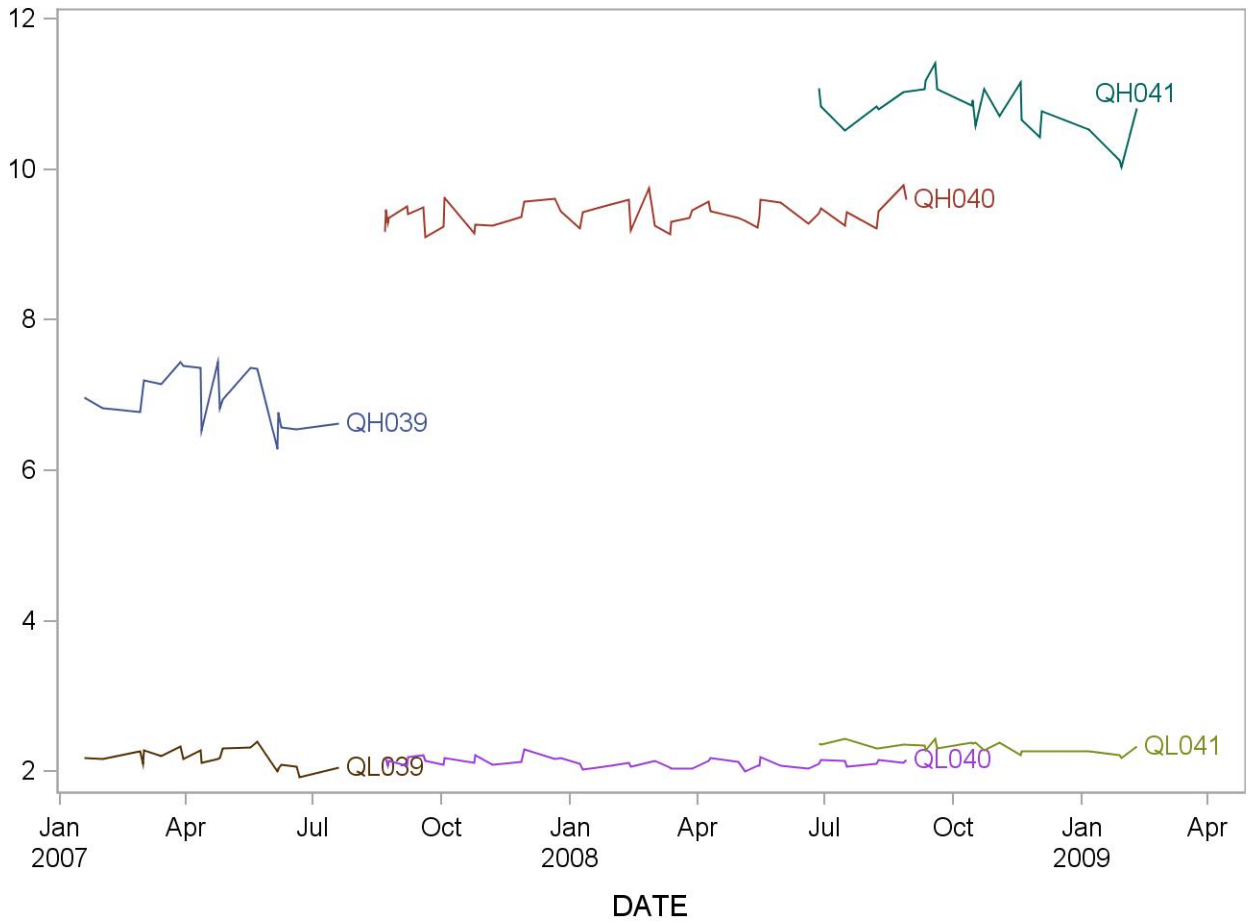
QL039	4	18JAN07	31JAN07	2.17800	0.06764	3.1
QL039	29	18JAN07	19JUL07	2.16950	0.12196	5.6
QH040	56	21AUG07	28AUG08	9.39692	0.21803	2.3
QL040	55	21AUG07	28AUG08	2.12290	0.06765	3.2
QH041	28	27JUN08	09FEB09	10.79840	0.32761	3.0
QL041	28	27JUN08	09FEB09	2.32131	0.06885	3.0



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QH039	4	18JAN07	31JAN07	0.12573	0.00326	2.6
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QL039	4	18JAN07	31JAN07	0.36801	0.00712	1.9
QL039	27	18JAN07	19JUL07	0.35466	0.02037	5.7
QH040	51	21AUG07	28AUG08	0.20538	0.01910	9.3
QL040	55	21AUG07	28AUG08	0.14450	0.01461	10.1
QH041	28	27JUN08	09FEB09	0.94023	0.03954	4.2
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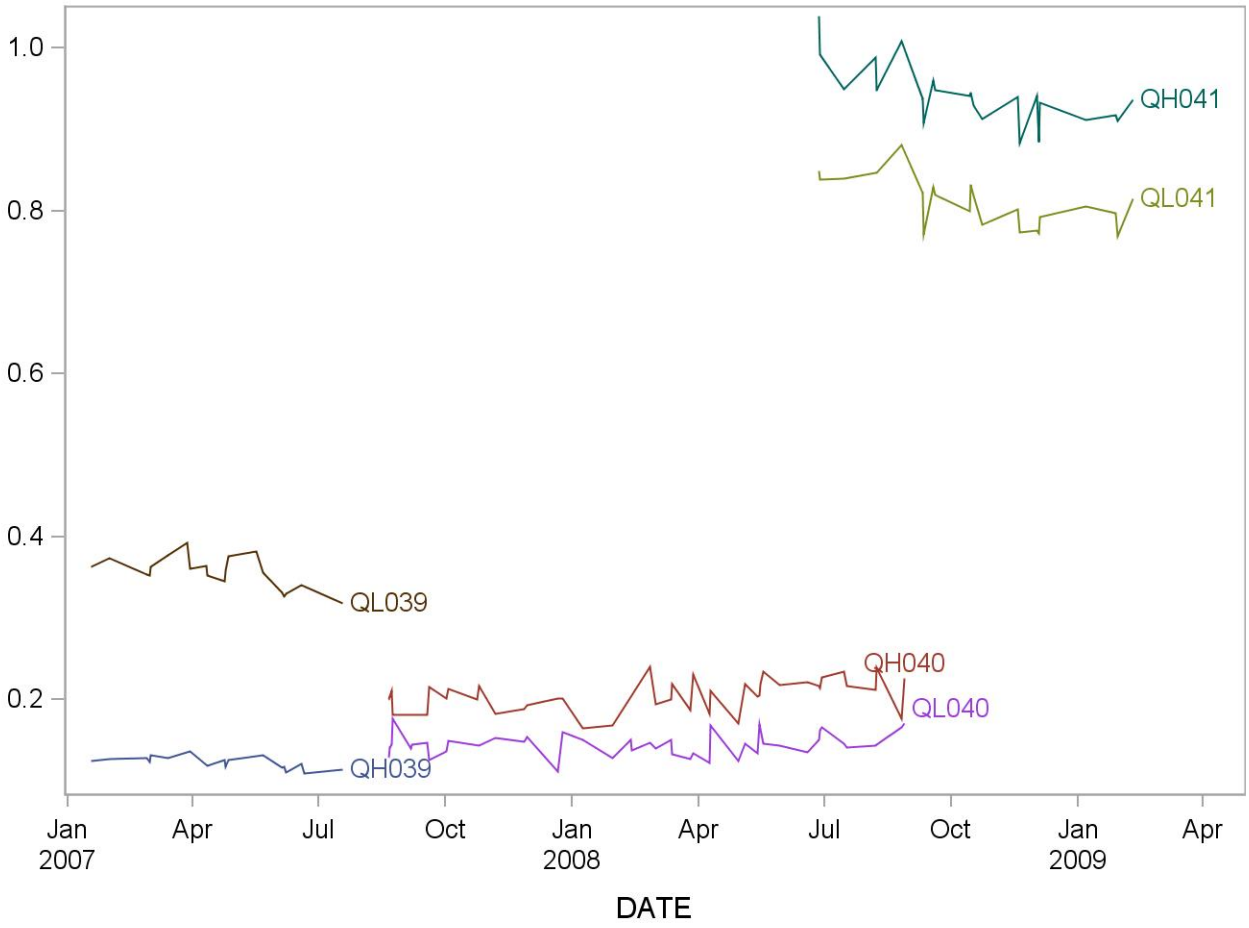

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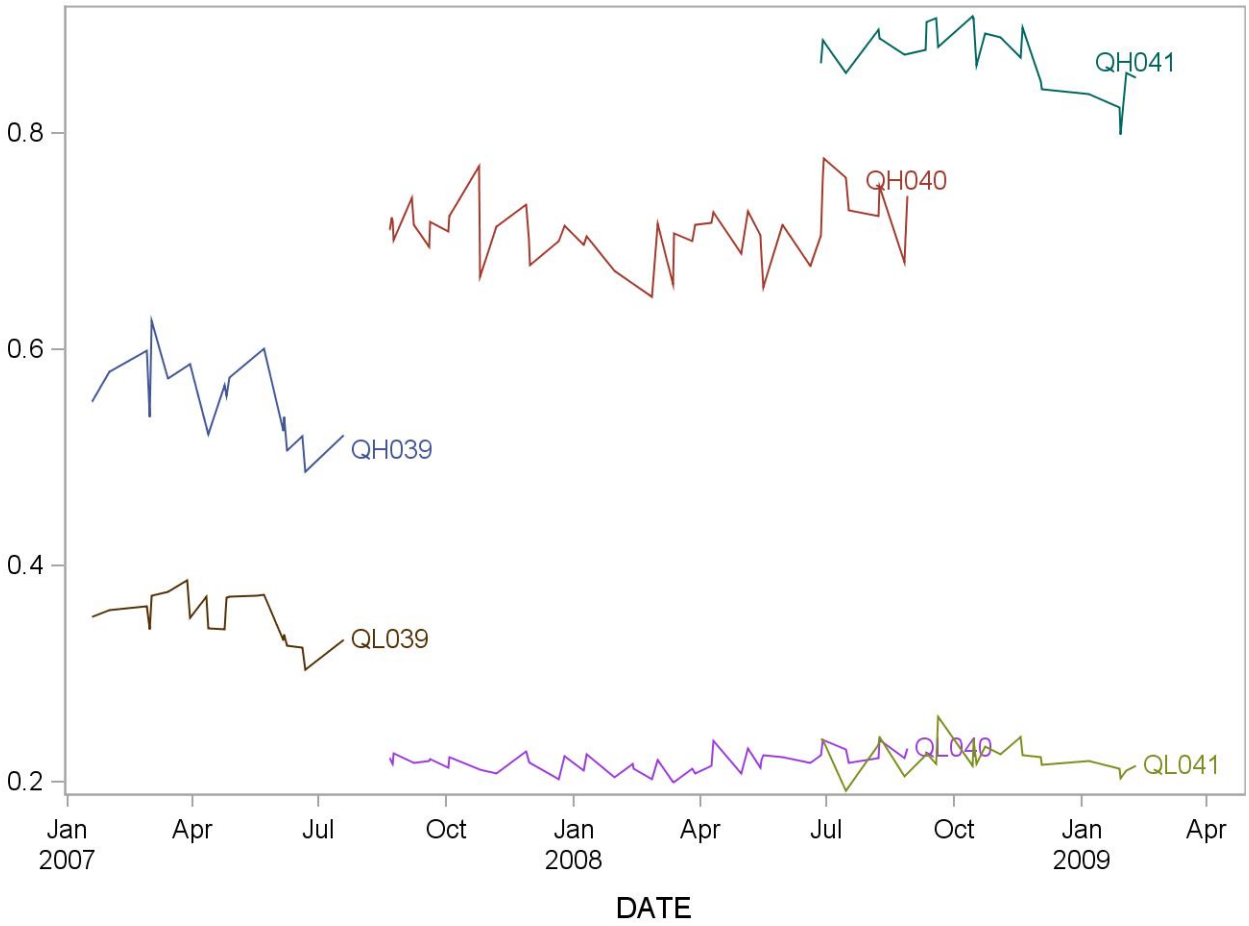
QL039	3	18JAN07	31JAN07	0.35532	0.01078	3.0
QL039	27	18JAN07	19JUL07	0.35137	0.02198	6.3
QH040	54	21AUG07	28AUG08	0.71118	0.02836	4.0
QL040	56	21AUG07	28AUG08	0.21843	0.01051	4.8
QH041	28	27JUN08	09FEB09	0.87194	0.03159	3.6
QL041	28	27JUN08	09FEB09	0.22536	0.01595	7.1



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QL039	4	18JAN07	31JAN07	0.16970	0.00553	3.3
QL039	29	18JAN07	19JUL07	0.16090	0.01426	8.9
QH040	51	21AUG07	28AUG08	0.63273	0.07903	12.5
QL040	54	21AUG07	28AUG08	0.37050	0.04670	12.6
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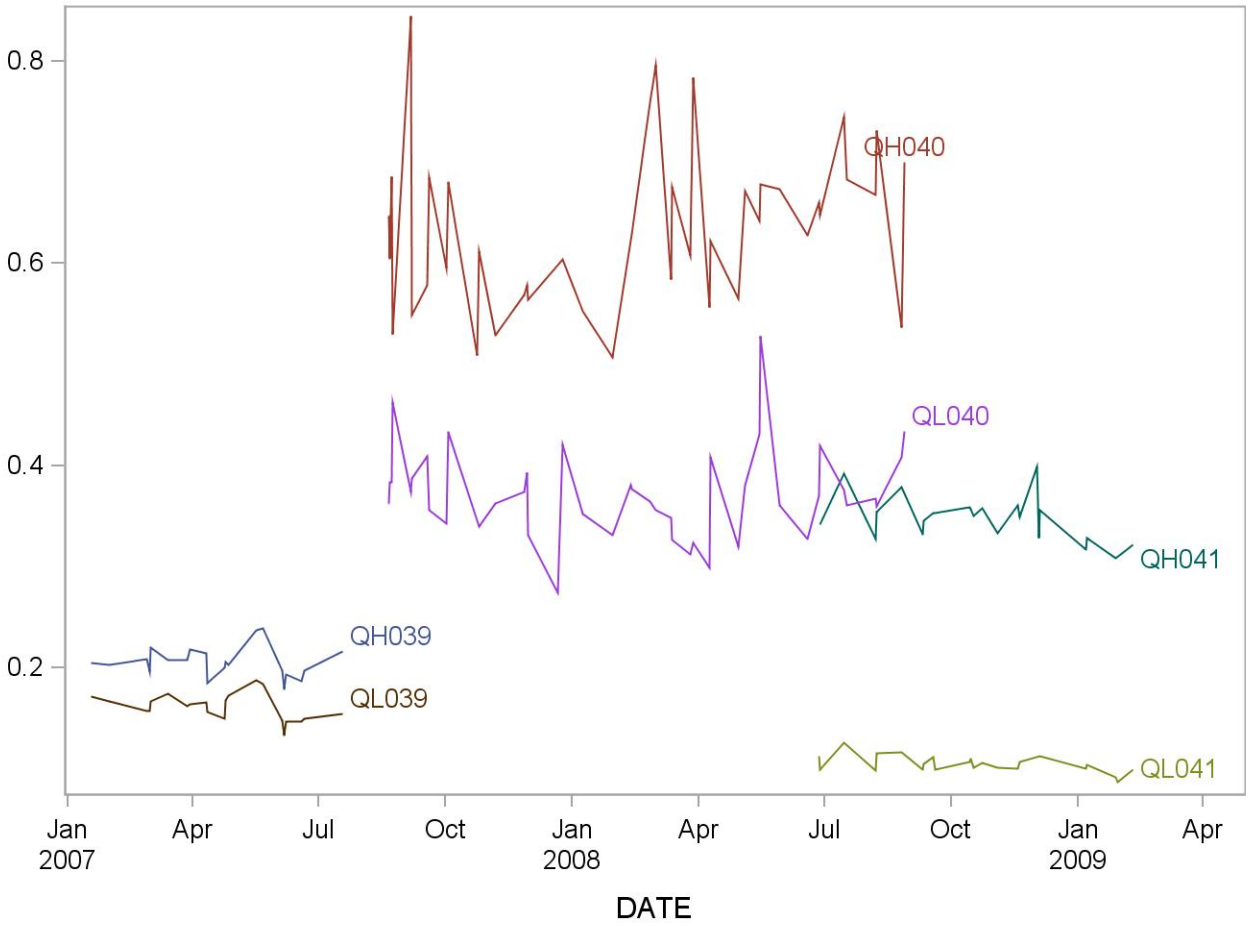

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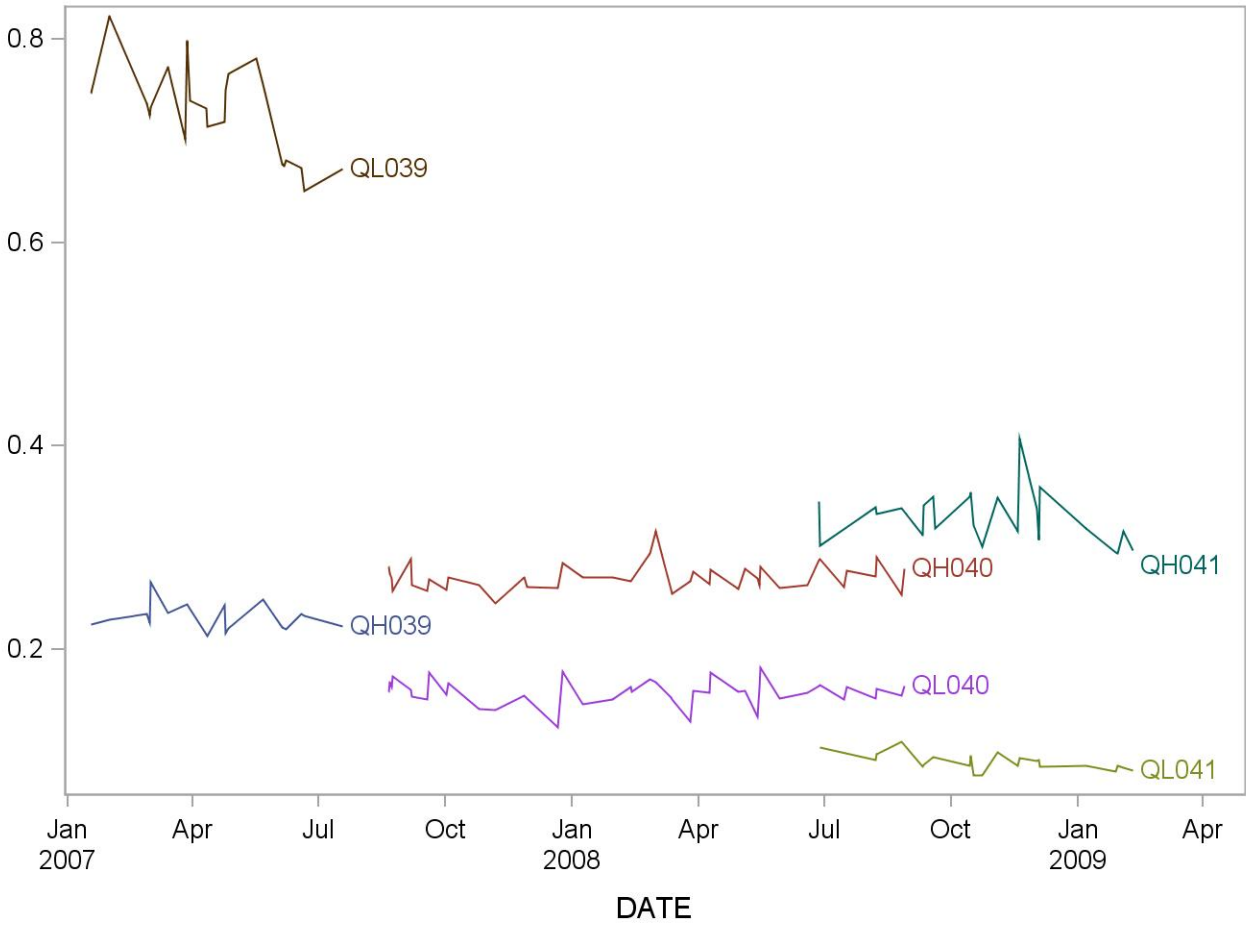
QL039	4	18JAN07	31JAN07	0.78449	0.07841	10.0
QL039	30	18JAN07	19JUL07	0.73082	0.05779	7.9
QH040	52	21AUG07	28AUG08	0.27054	0.01490	5.5
QL040	53	21AUG07	28AUG08	0.15702	0.01210	7.7
QH041	28	27JUN08	09FEB09	0.32962	0.02574	7.8
QL041	26	28JUN08	09FEB09	0.08955	0.00851	9.5



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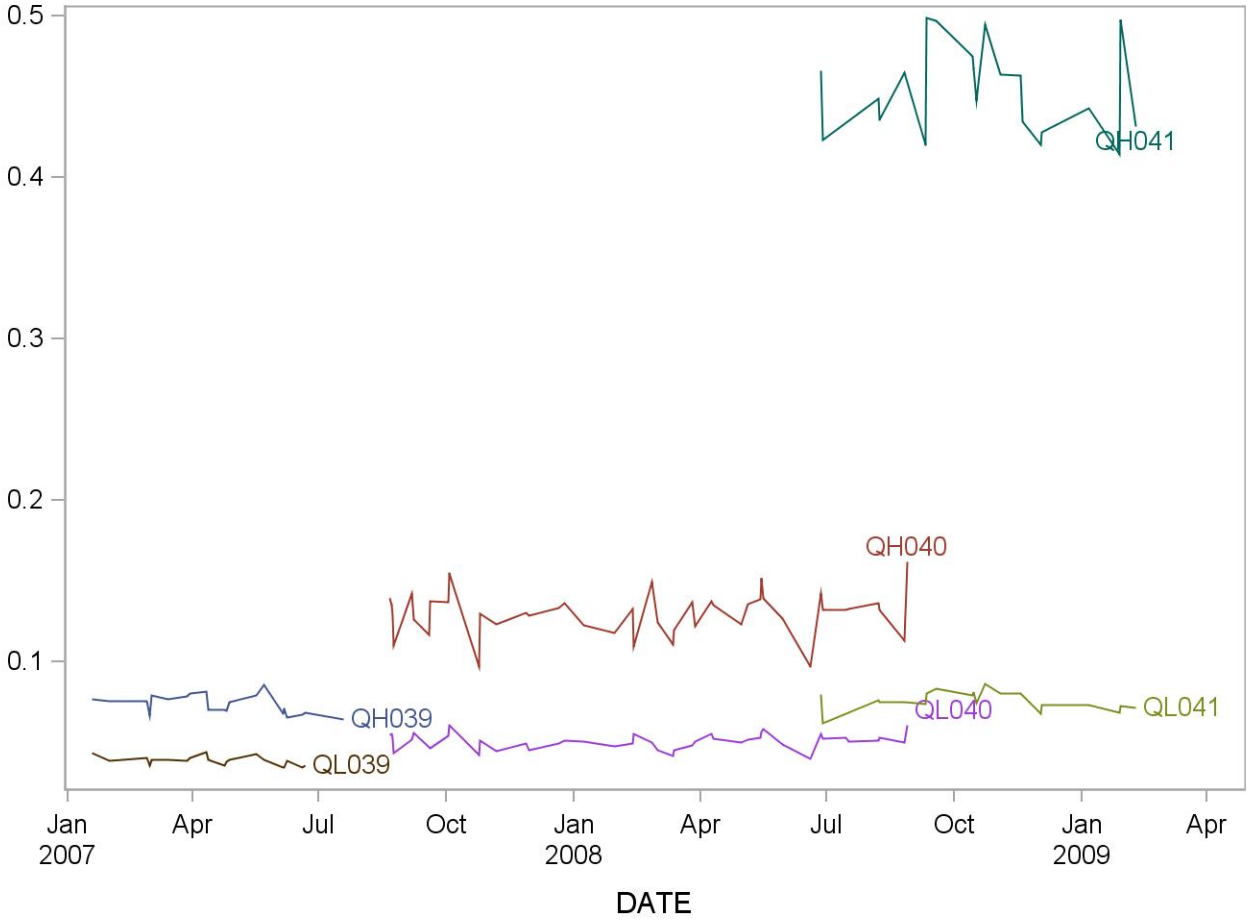
QL039	4	18JAN07	31JAN07	0.04061	0.00309	7.6
QL039	28	18JAN07	21JUN07	0.03831	0.00305	8.0
QH040	55	21AUG07	28AUG08	0.12923	0.01521	11.8
QL040	55	21AUG07	28AUG08	0.05031	0.00541	10.8
QH041	26	27JUN08	09FEB09	0.45166	0.02735	6.1
QL041	26	27JUN08	09FEB09	0.07540	0.00556	7.4



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QH039	4	18JAN07	31JAN07	0.09376	0.00977	10.4
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QL039	4	18JAN07	31JAN07	0.09003	0.00761	8.5
QL039	29	18JAN07	19JUL07	0.08892	0.01002	11.3
QH040	55	21AUG07	28AUG08	0.15001	0.02226	14.8
QL040	55	21AUG07	28AUG08	0.06653	0.00618	9.3
QH041	30	27JUN08	09FEB09	0.30829	0.03541	11.5
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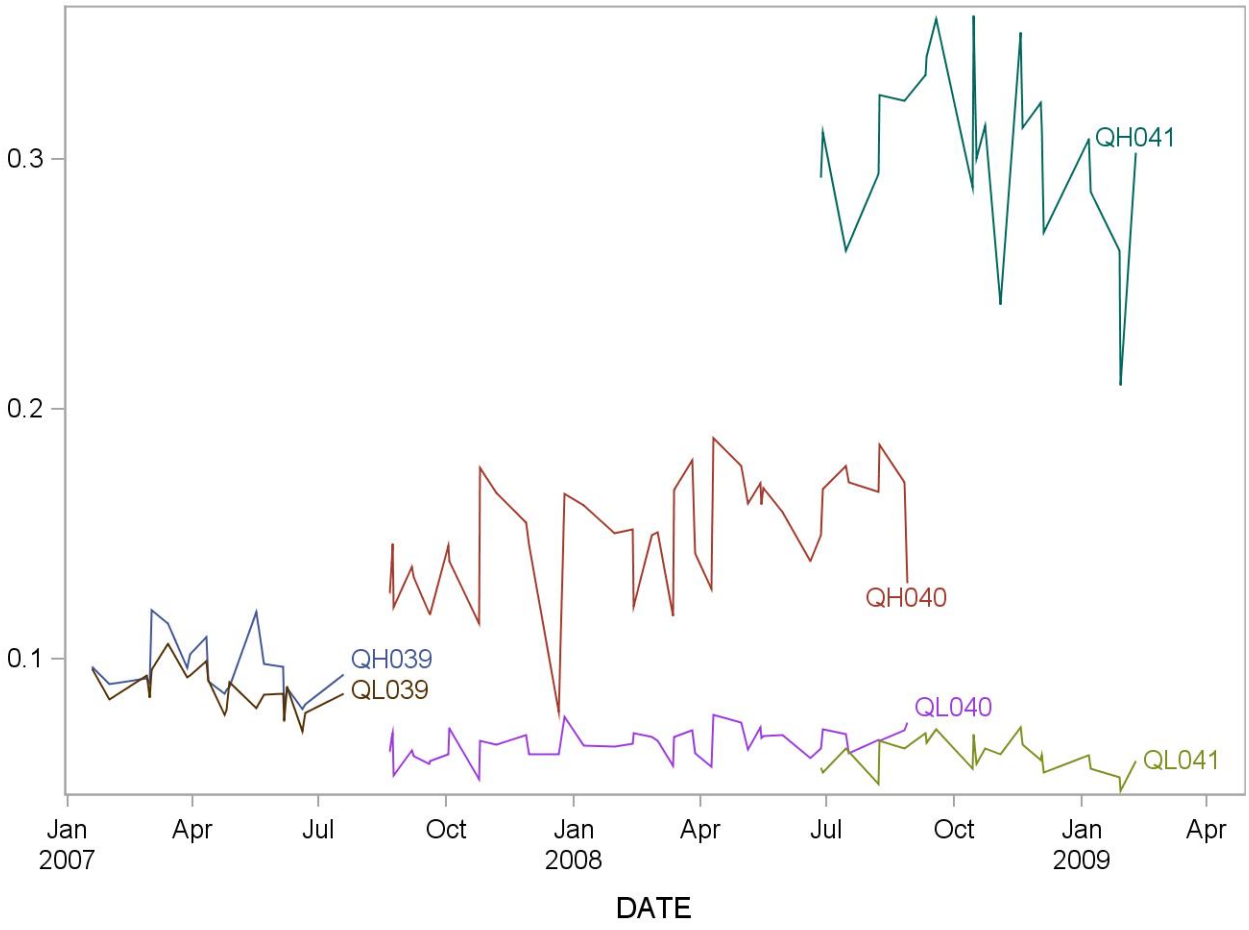

Reference List

1. Blount, B. C.; Kobelski, R. J.; McElprang, D. O.; Ashley, D. L.; Morrow, J. C.; Chambers, D. M.; Cardinali, F. L. Quantification of 31 volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography-mass spectrometry. *J Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2006,** *832* (2), 292-301.

2. Chambers, D.M.; Blount, B.C.; McElprang, D.O.; Waterhouse, M.G.; Morrow, J.C. Picogram Measurement of Volatile n-Alkanes (n-hexane through n-dodecane) in Blood using Solid-Phase Microextraction to Assess Non-occupational Petroleum-Based Fuel Exposure *Anal. Chem.* **2008** (80), 4666–4674.

3. Chambers, D. M.; McElprang, D. O.; Mauldin, J. P.; Hughes, T. M.; Blount, B. C. Identification and elimination of polysiloxane curing agent interference encountered in the quantification of low-picogram per milliliter methyl tert-butyl ether in blood by solid-phase microextraction headspace analysis. *Anal. Chem.* **2005,** *77* (9), 2912-2919.

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