

Troubleshooting Contamination in Integrated Systems

This document outlines a strategy for troubleshooting contamination in integrated systems.

NOTE: For general information and a starting point to integrated system troubleshooting, see [Integrated System Troubleshooting](#), located in the Service Knowledge Center of the Portal.

NOTE: See also [Controlling Contamination in LC/MS Systems](#) and [Routine Cleaning for the ACQUITY UPLC System](#) or [Routine Cleaning for Purification Systems](#), located on Waters.com.

It contains information on:

- [Using a systematic process](#), page 1
- [Gathering information about contamination](#), page 4
- [Locating the source of contamination](#), page 5
- [Troubleshooting the MS System](#), page 9
- [Testing for contamination](#), page 10
- [Cleaning contamination](#), page 11
- [Causes of contamination](#), page 15

Using a systematic process

Troubleshooting contamination is a complex process that requires time and experimentation. A successful troubleshooting strategy includes the tasks listed in [Table 1](#).

Table 1: Contamination troubleshooting tasks

Troubleshooting task	Description
Gather information See Gathering information about contamination , page 4	Before trying to remove system contamination, you must determine its nature and scope: what type of contamination it is, how it occurred, and how extensive it is. Taking the time to investigate it fully can shorten the overall troubleshooting process.
Test See Testing for contamination , page 10	Throughout the process you must repeatedly test the data to check troubleshooting progress. It is important to set up testing conditions before starting to troubleshoot.
Locate the source See Locating the source of contamination , page 5	Eliminating the source of contamination is an essential part of troubleshooting contamination. If you attempt to clean the system before performing this step, contamination will return and the cleaning process will have to be repeated. Knowledge of chemistry is helpful in locating the source of contamination.
Eliminate/clean See Cleaning contamination , page 11	The cleaning process and the choice of cleaning mixtures should be based on information gathered about the type of contamination found. The solubility properties of the contaminants are important for the choice of solvents and pH. NOTE: Do not attempt to clean the system before locating and eliminating the source of contamination.

Figure 1 outlines a systematic approach to troubleshooting contamination in an LC/MS system.

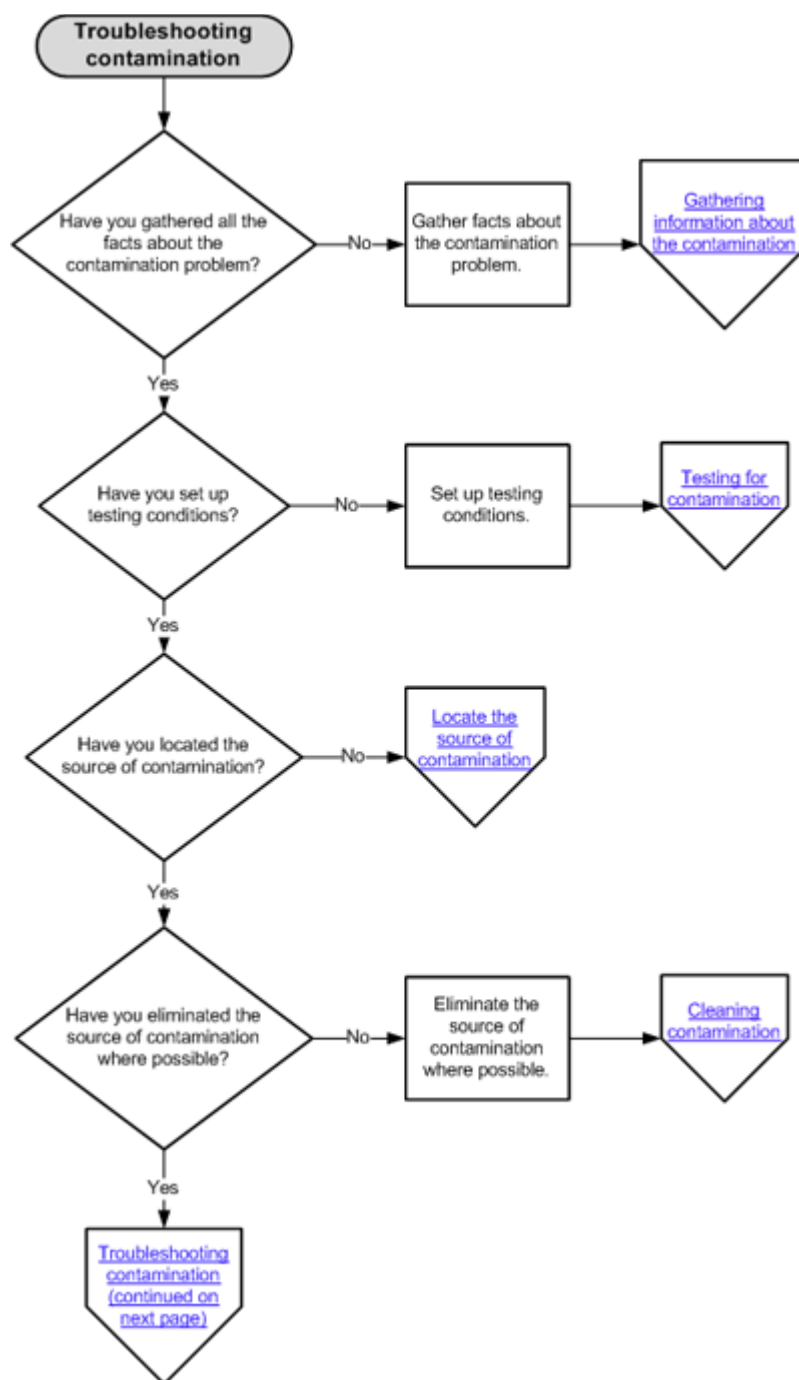


Figure 1 - Troubleshooting contamination

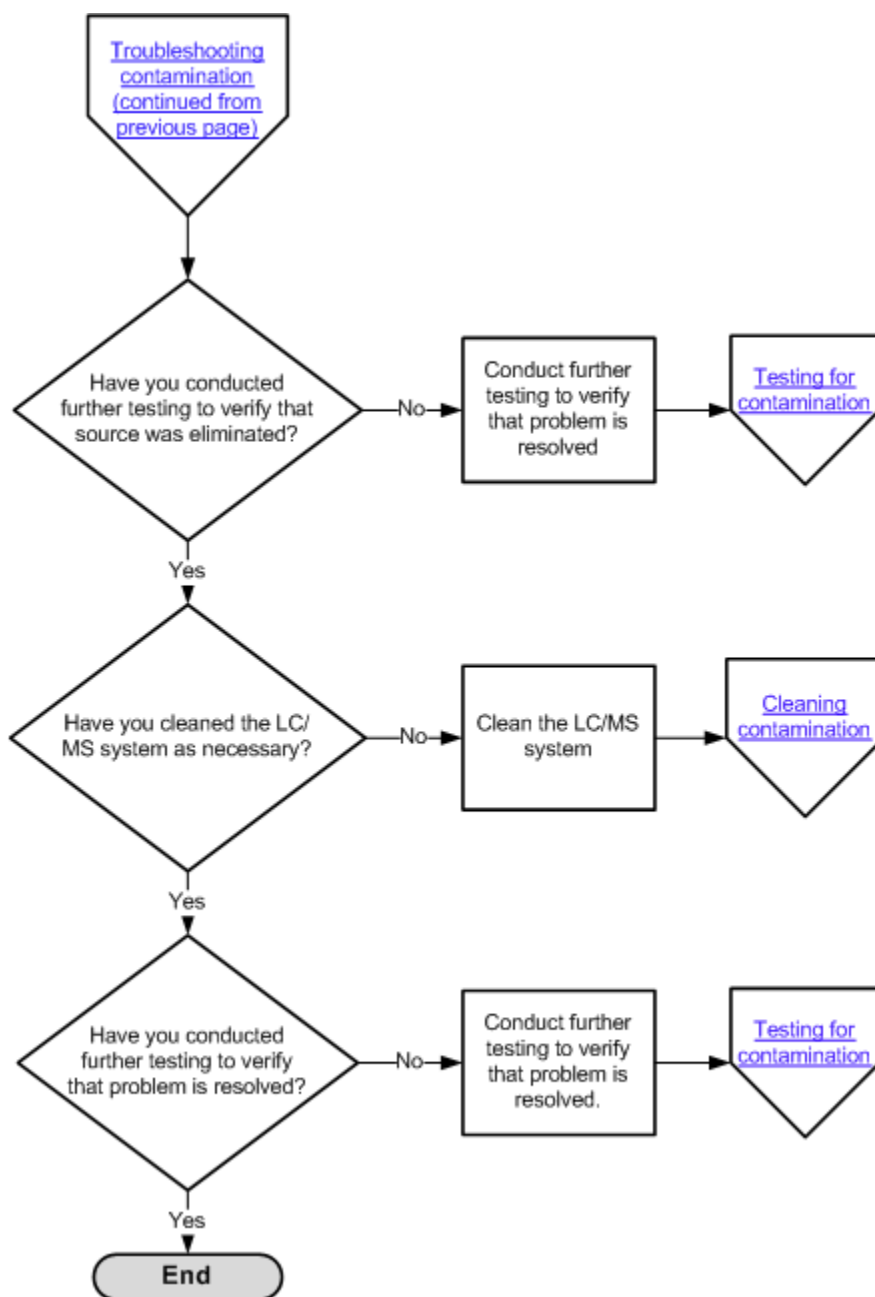


Figure 1 - Troubleshooting contamination (continued)

Gathering information about contamination

Figure 2 outlines the information-gathering step of the process.

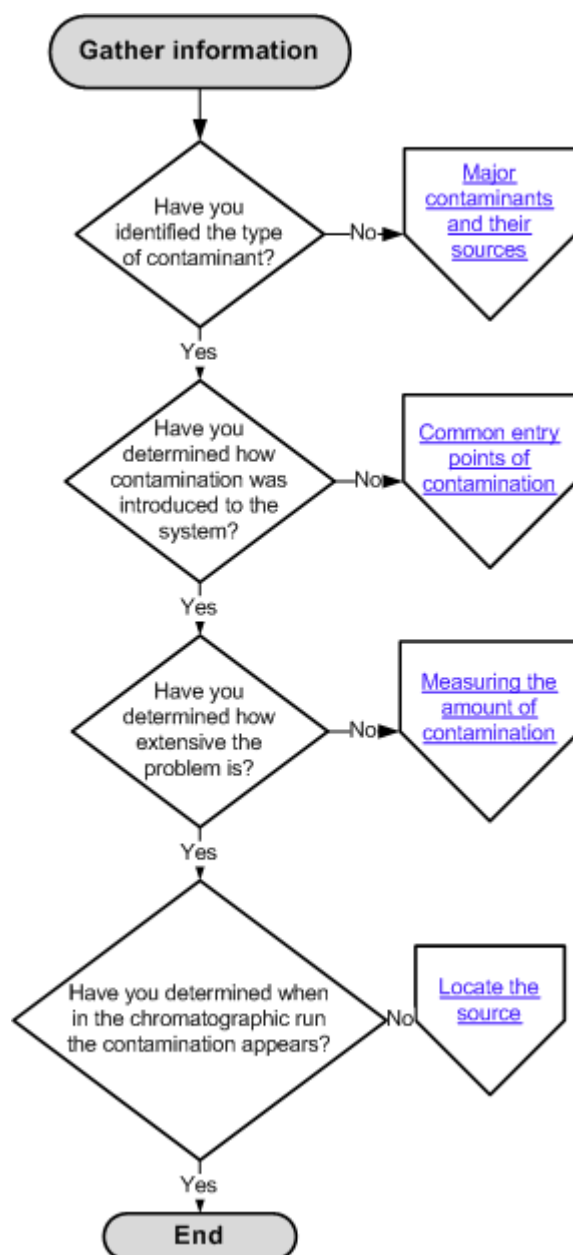


Figure 2 - Gathering information about contamination

Locating the source of contamination

Figure 3 outlines the process for locating the source. Figure 4 shows the process for locating the source of contamination in an LC system.

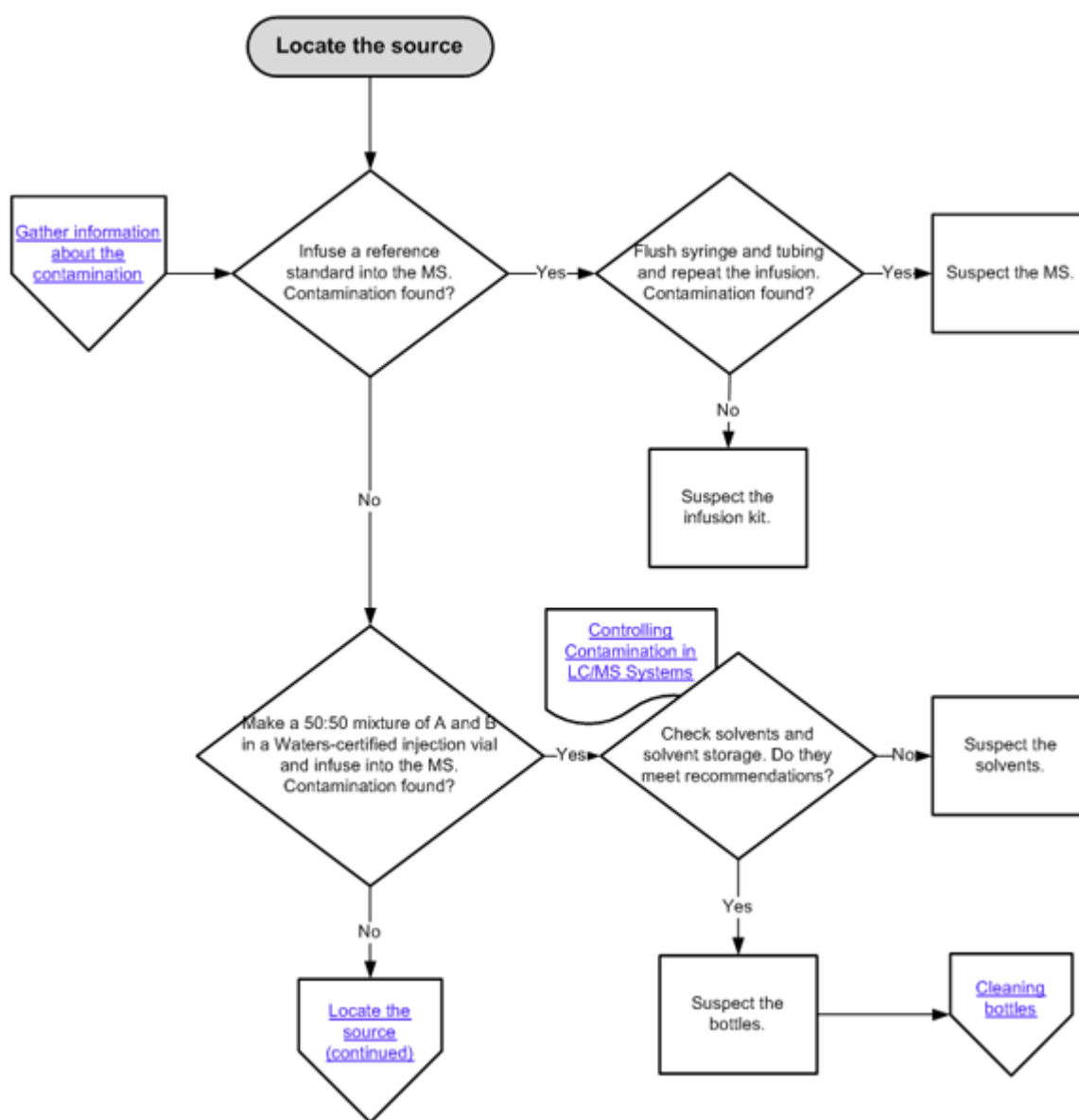


Figure 3 - Locating the source of contamination

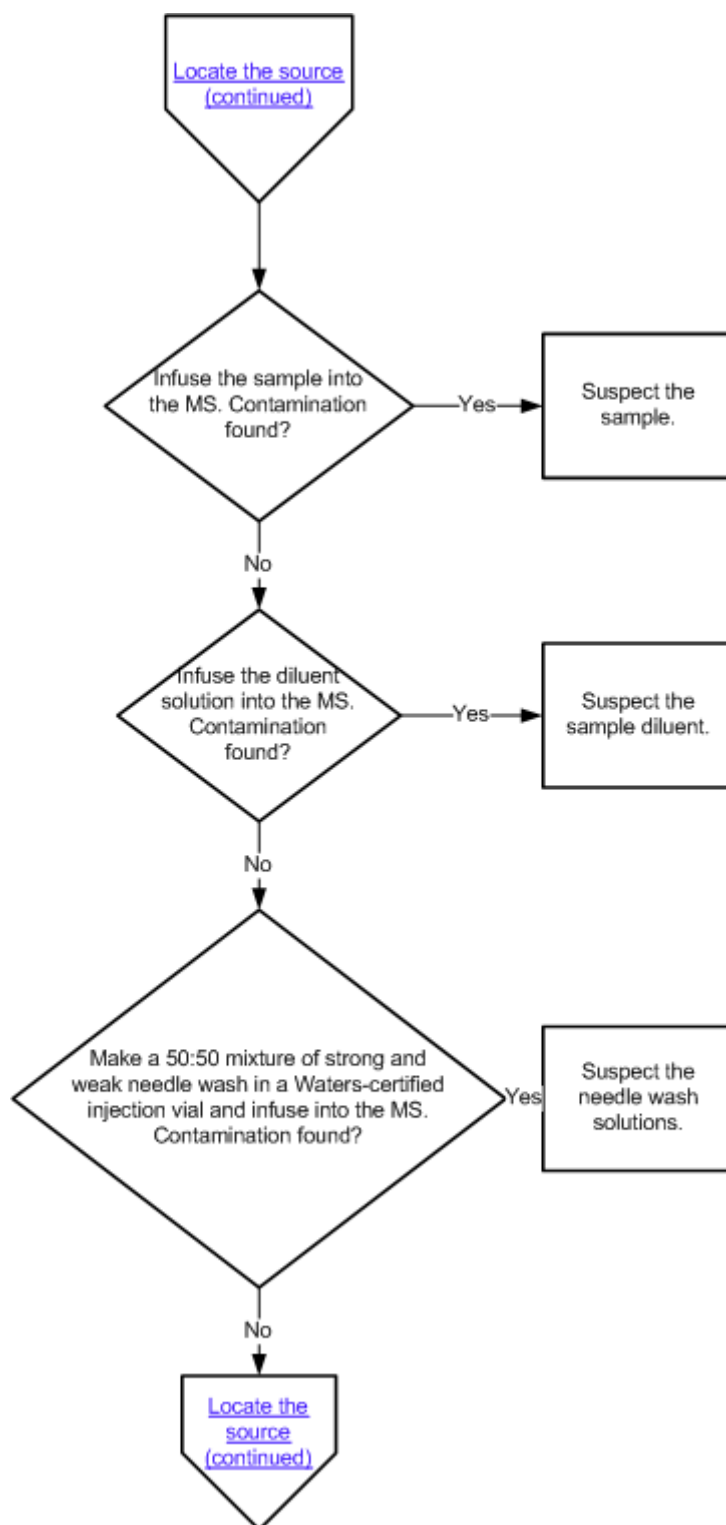


Figure 3 - Locating the source of contamination (continued)

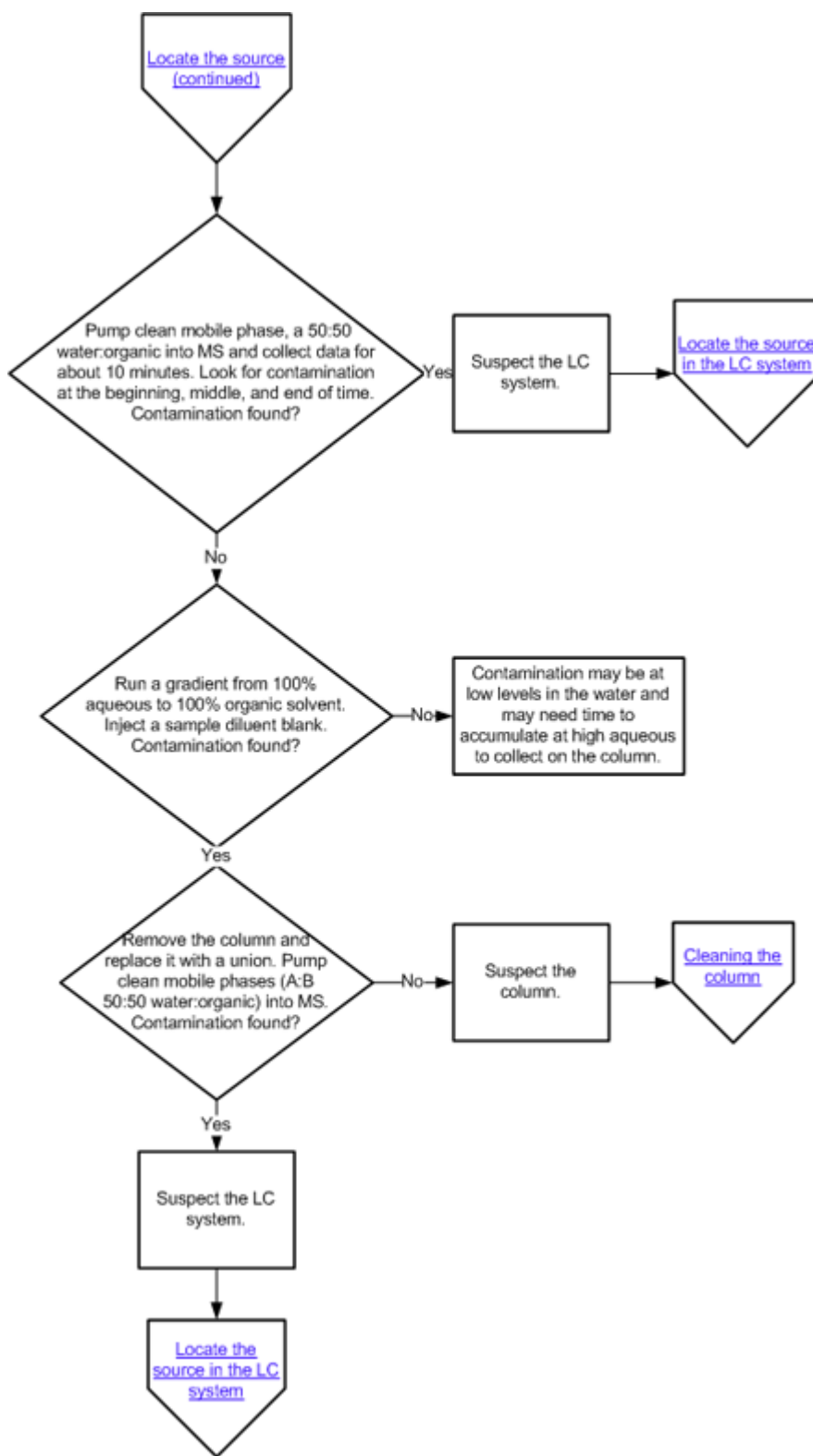


Figure 3 - Locating the source of contamination (continued)

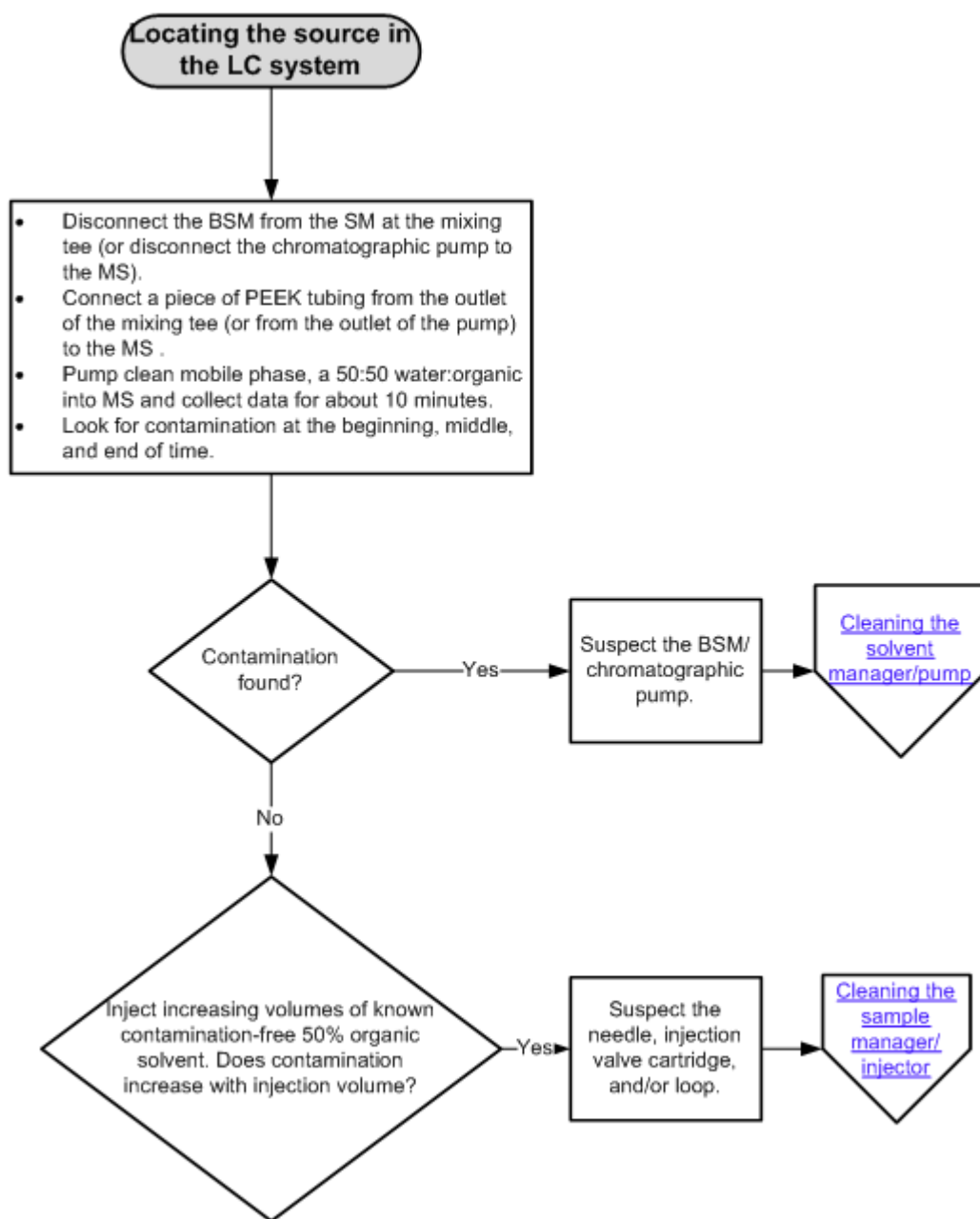


Figure 4 - Locating the source of contamination in the LC System

Troubleshooting the MS System

If troubleshooting the liquid chromatography system does not yield the location of contamination, the likely source is the mass spectrometry system.

CAUTION: *TAKE CARE NOT TO WASTE TIME AND RESOURCES ATTEMPTING TO REMOVE TYPICAL BACKGROUND NOISE. THE SENSITIVE NATURE OF MS SYSTEMS DICTATES THAT SOME DEGREE OF CHEMICAL BACKGROUND IS A CONSTANT. IN ADDITION, DIFFERENT TYPES OF MS SYSTEMS HAVE DIFFERENT DEGREES OF SENSITIVITY. FOR EXAMPLE, YOU WILL SEE A HIGHER BACKGROUND IN A MORE SENSITIVE INSTRUMENT.*

Check the front end components

Likely locations for MS contamination are front end components:

- ESI probe (probe tip, capillary, unions)
- Sample cone
- Lockspray baffle
- Ion source block
- Source enclosure
- PEEK tubing connecting column outlet to API source
- Components of the integral flow divert/injection valve (if fitted)
- Throttle valve (if fitted)
- PEEK support block
- First ion guide (or hex)
- LC tubing
- Nitrogen gas tubing
- Nitrogen gas source (e.g., generator)

Remove, clean or replace, and test the components

Remove, clean or replace, and test each of these components one at a time. If contamination still exists, the MS components may have become recontaminated after cleaning. To avoid this problem, clean and replace all suspected parts simultaneously. Make a note of which parts you have replaced, and retain the replaced parts for testing to isolate contamination.

Testing for contamination

To simplify interpretation of data, follow these guidelines for testing and collecting data.

Table 2: Test parameter guidelines

Test material	Recommendation
Infusion kit: • syringe • tubing • injection port fitting	Ensure that all parts are clean. Flush with known clean solvents.
Connecting tubing	Use new if possible.
Solvents for testing	Use Waters-recommended solvent brands. See Controlling Contamination in UPLC/MS and HPLC/MS Systems .
Solvent bottles	Put flush solvents in known clean bottles.

Measuring the amount of contamination

Part of the information gathering process is determining how extensive the contamination is:

1. Is the change from “normal” a small change (2X) or a large change (>10X)?
2. Is there ion suppression and loss of sensitivity?

Many factors can affect the apparent magnitude (total counts) of contamination. Use the recommended test conditions when measuring the amount of contamination. Keep all conditions the same for each test.

Table 3: Test measuring guidelines

Factor affecting amount of contamination	Suggested test condition
MS tuning parameters (affect sensitivity)	Tune the MS on a reference solution and use that tune file for all of the testing.
Composition of the water-organic mixture (affects desolvation). The presence or absence of acid helps ionization.	For ESI+, use one of the following mixtures: • 50:50 mixture of water-organic • 50:50 A:B with 0.1% formic acid
Type of source	Use the same source throughout the testing.
Flow rate	Use a low flow rate when pumping mobile phase.
Data acquisition rate and mass range	Acquire at a scan rate of 1 per second over a wide mass range (e.g., 50-2000) in continuum mode.
Data presentation	Submit screen captures with technical inquiries and requests for help.

Table 3: Test measuring guidelines (continued)

Factor affecting amount of contamination	Suggested test condition
Chromatograms	Show the entire chromatogram as a TIC. Enlarge the problematic region.
Spectra	Show spectra of the problematic region of the chromatogram. Show single-scan spectra. Then combine the same number of spectra (20 is a good number), and the points across a chromatographic peak. Do not combine hundreds of spectra or sum minutes of data. For ease of comparison, link the vertical axes.
Methods (including solvents, column, sample) and acquired data	Supply raw data if possible when requesting help.

Cleaning contamination

Universal cleaning procedures have not been developed. Cleaning requires flushing until the contamination is minimal. This can take hours to days.

NOTE: For more information, see [Routine Cleaning for the ACQUITY UPLC System](#) or [Routine Cleaning for Purification Systems](#).

The cleaning process and the choice of cleaning mixtures should be based on information gathered about the type of contamination found (see [Gathering information about contamination, page 4](#)). The solubility properties of the contaminants are important for the choice of solvents and pH.

NOTE: Do not attempt to clean the system before locating and eliminating the source of contamination.

After cleaning, retest to confirm that the problem has been resolved. Be sure to retest under the same conditions that were used for the original tests so that you can compare the magnitude of the background. To simplify the process, use a single bottle of 50:50 water:organic.

Cleaning mixtures

When choosing a cleaning mixture, consider the solubility properties of the contaminants found during the step [Locating the source of contamination, page 5](#).

Make sure the cleaning solvents are Waters-recommended brands (see [Controlling Contamination in UPLC/MS and HPLC/MS Systems](#)) or the highest-purity solvents available. Test the solvent before using it.

For information on:

- recommended cleaning mixtures for LC systems, see [Table 4](#)
- recommended cleaning mixtures for MS systems, see [Table 5](#).

Table 4: Recommended Cleaning Mixtures for LC

LC MIXTURE ^A	PURPOSE	MIXTURE COMPOSITION	CAUTIONS AND COMMENTS
1	General purpose solution for nanoACQUITY or other applications where use of high-pH mobile phase is not advisable	100% 2-propanol (isopropyl alcohol, or IPA)	NA
2	"Universal" wash solution for high background	• 25% acetonitrile • 25% methanol • 25% 2-propanol • 25% water • 0.2% formic acid (for acid conditions) or 0.2% hydroxide (for basic conditions)	• USE FORMIC ACID HIGH CONCENTRATIONS IN STRONG NEEDLE WASH FOR ACID CONDITIONS. USE AMMONIA HYDROXIDE FOR BASIC CONDITIONS. • DO NOT USE HYDROXIDE IN NANOACQUITY.
3	Use to remove PEG and amide contamination	• 50% acetonitrile • 49% water • 1% ammonium hydroxide	• HIGH-PH WASH DISSOLVES SILICA ABOVE PH=10. IN SYSTEMS WITH FUSED SILICA AND GLASS COMPONENTS, SUCH AS NANOACQUITY, DO NOT USE MIXTURE 3. • AFTER CLEANING WITH ACID OR BASE, FLUSH WITH ULTRAPURE WATER UNTIL THE PH IS NEUTRAL (ABOUT PH=7) BEFORE CONNECTING TO A DETECTOR.
4	• To remove most contaminants • For cleaning metal components only For more information, see Routine Cleaning for the ACQUITY UPLC System or Routine Cleaning for Purification Systems.	• 30% phosphoric acid • 70% water	• USE AS LAST RESORT • DO NOT USE MIXTURE 4 WITH ORGANIC SOLVENTS. • DO NOT USE MIXTURE 4 WITH NANOACQUITY. • DO NOT USE MIXTURE 4 TO CLEAN SEAL-WASH LINES. • REMOVE HASTELLOY SINKERS, IF USED, BEFORE CLEANING WITH PHOSPHORIC ACID • AFTER CLEANING WITH ACID OR BASE, FLUSH WITH ULTRAPURE WATER UNTIL THE PH IS NEUTRAL (ABOUT PH=7) BEFORE CONNECTING TO A DETECTOR.

A. CAUTION: DO NOT INTRODUCE ANY CLEANING SOLUTIONS INTO THE MS SYSTEM OR COLUMN.

Table 5: Recommended Cleaning Mixtures for MS

MS MIXTURE	PURPOSE	MIXTURE COMPOSITION	CAUTIONS AND COMMENTS
1	To remove hydrophilic contaminants	Water	NA
2	To remove hydrophobic contaminants	Organic solvent such as 2-propanol, methanol, or acetonitrile	2-propanol is a better solvent for many things than methanol or acetonitrile
3	To remove most contaminants	• 50% organic solvent • 50% water	NA
4a	Strong acid wash	• 45% acetonitrile or methanol • 45% water • 10% formic acid	• Do not sonicate PEEK components or T-WAVE assemblies in mixture 4. • After sonicating with mixture 4, sonicate in mixture 3. • Use formic acid high concentrations in strong needle wash for <i>acid</i> conditions. Use ammonia hydroxide for <i>basic</i> conditions.
4b	Strong basic wash	• 45% acetonitrile or methanol • 45% water • 10% hydroxide	• Do not sonicate PEEK components or T-WAVE assemblies in mixture 4. • After sonicating with mixture 4, sonicate in mixture 3. • Use formic acid high concentrations in strong needle wash for <i>acid</i> conditions. Use ammonia hydroxide for <i>basic</i> conditions.
5	• For cleaning metal components only • Used to remove contamination such as hydrocarbons, oil, or grease	Chlorinated solvents, hexane, or acetone	• Do not sonicate PEEK components or T-WAVE assemblies in mixture 5. • After sonicating with mixture 5, sonicate in 2-propanol (required if mixture 5 is not miscible in water), and then sonicate in mixture 3.

Cleaning LC Systems

General guidelines

To clean contamination in LC systems, use the highest-purity solvent mixtures and make sure solvent containers are clean. If you know what the contaminant is, use the cleaning mixture in which it is most soluble. See [Table 4](#) on page 12 for recommended LC cleaning mixtures.

Flush the system component with a high-organic solvent such as 100% acetonitrile, test for contamination, and repeat the procedure until the background is down to an acceptable level.

After using any wash, rinse with 50% acetonitrile or mobile phase to remove the cleaning solution. If you use mixture 4, pump ultrapure water through the system until the pH is neutral (about pH=7).

WARNING: ALWAYS USE SAFE LABORATORY PRACTICES WHEN WORKING WITH SOLVENTS AND WASH SOLUTIONS. KNOW THE CHEMICAL AND PHYSICAL PROPERTIES OF THE SOLVENTS AND SOLUTIONS. REFER TO THE MATERIAL SAFETY DATA SHEET (MSDS) FOR EACH SOLVENT AND SOLUTION IN USE.

WARNING: TO PREVENT INJURY, ALWAYS USE EYE PROTECTION AND GLOVES WHEN HANDLING SOLVENTS OR CLEANING MIXTURES.

CAUTION: DISCONNECT THE LC SYSTEM FROM THE COLUMN AND DETECTOR BEFORE CLEANING.

CAUTION: SOLVENTS MUST BE OF THE HIGHEST PURITY FOR CLEANING AND UPLC OR HPLC USE.

NOTE: For more information on solvent recommendations and cautions, see your system operator's guide.

NOTE: For more information on cleaning procedures, see [Routine Cleaning for the ACQUITY UPLC System](#) or [Routine Cleaning for Purification Systems](#).

Cleaning the solvent manager/pump

For cleaning mixtures, see [Table 4](#).

1. Remove the column and disconnect the detectors and the MS.
2. Flush the solvent manager with large volumes (hundreds of mLs) of cleaning mixtures
 - a. To speed up the flushing, create backpressure greater than 500 psi by installing an old column and restrictor or length of narrow-ID (0.0025") PEEK tubing. Vary the backpressure (change the flow rate) by programming flow step gradients.
 - b. Flush A1, A2, B1, and B2 by running 50:50 A:B pairs. This will also clean the degasser and tubing.
3. While flushing, occasionally retest the contamination level to measure progress.
4. When finished, return the system to the customer's clean mobile phases and check.

Cleaning the sample manager/injector

For cleaning mixtures, see [Table 4](#).

1. Prime the syringes with the cleaning mixture for 10 cycles.

2. Do many full loop injections with maximum number of overfills using the cleaning mixture in a sample vial.

Cleaning the column

For cleaning mixtures, see [Table 4](#).

1. Clean the column with either a series of water-organic gradients from 90:10 to 10:90 water:organic, or a series of step gradients. Running at 100% organic may work; however, not all contaminants are soluble in 100% organic.
2. Replace the column.

NOTE: For more information, see the column care and use manual appropriate to your system.

Cleaning bottles

For cleaning mixtures, see [Table 4](#).

You can clean the bottles or replace them with new borosilicate ones that have been cleaned. To ensure bottles are clean:

1. Use borosilicate (Pyrex or Schott bottles). Never use the brown bottles that the solvent comes in as mobile phase bottles.
2. Clean bottles with only LC/MS grade water and organic solvents.

Causes of contamination

This section lists some common transmission methods and sources of contamination, along with a partial list of contaminants.

This section lists:

- [Common entry points of contamination, page 15](#)
- [Common sources of contamination, page 17](#)

Common entry points of contamination

When contamination occurs, it is important to find out how it was introduced to the system. Table lists some common entry points on new and used systems.

Table 6: Areas to check when contamination appears on a new system

Area to check	Possible cause	Corrective action
Solvents	Solvents are not highest purity or do not otherwise meet requirements.	Use Waters-recommended solvents. See Controlling Contamination in UPLC/MS and UPLC/MS Systems .

Table 6: Areas to check when contamination appears on a *new* system (continued)

Area to check	Possible cause	Corrective action
Solvent bottles	Bottles are not clean (or were washed in dishwasher), are not borosilicate glass (Pyrex), or are plastic.	Use clean, Waters-recommended bottles. See Controlling CHntamination in UPLC/MS and UPLC/MS Systems .

Table 7: Areas to check when contamination appears on a *used* system

Area to check	Possible cause	Corrective action
Solvents	A new source of solvents was used that is not as pure as the old source.	Use Waters-recommended solvents. See Controlling CHntamination in UPLC/MS and UPLC/MS Systems .
	Old solvents or mobile phases have degraded, possibly adsorbing contaminants from the air or bottles.	Replace old solvents and mobile phases with Waters-recommended brands from Waters-recommended sources. See Controlling CHntamination in UPLC/MS and UPLC/MS Systems .
Column	A new column containing contaminants was used.	Flush the column at 85% organic-water or 100% organic to condition and clean it.
	An old column was used, but it has accumulated contaminants from the mobile phase or sample and is now slowly releasing them.	1. Replace the column. 2. Flush the new column at 85% organic-water or 100% organic to condition and clean it.
Sample or mobile phase	A new method with different samples and mobile phases was used.	Investigate/troubleshoot what has changed in the method.
User	Inexperienced user	Process of elimination required. See Locate the source .
Recent maintenance	A PM was recently performed.	Suspect new parts or poor technique. Process of elimination required. See Locate the source .
Inline filter		
Column stabilizer		

Common sources of contamination

NOTE: For more information on major contaminants, see [PEG master list](#) and [Background Ion Master List](#).

Table 8: Common sources of contamination

Potential sources	Examples	Possible contaminants
Environmental	Air nitrogen compressed air	- Siloxanes - Phthalates - Hydrocarbons
Personal care products	- Hand cream - Antiperspirant	- Polyethylene glycol (PEG) - Siloxanes
Solvents	- Acetonitrile - Methanol	- Polyethylene glycol (PEG) - Adduct ions, e.g., sodium (Na^+, $m/z=23$), potassium (K^+, $m/z=39$), iron (Fe^{+3}, $m/z=55$), ammonium (NH_4^+, $m/z=18$) - Solvent clusters
	Water	- Microbial or algae growth - Salt adduct ions, e.g., sodium $m/z+23$ - Organic ions from plasticizers, etc.
Additives	- Formic acid - Acetic acid - Ammonium hydroxide - Trifluoroacetic acid (TFA)	- Adduct ions, e.g., sodium (Na^+, $m/z=23$), potassium (K^+, $m/z=39$), iron (Fe^{+3}, $m/z=55$) - Ammonium (NH_4^+, $m/z=18$) TFA ($m/z=114$)
Standards	Mass spectrometer reference standard	- Reserpine $m/z = 609$ - GFP (glu-fibrinopeptide) $m/z = 785.8$ - Polyethylene glycol (PEG), delta $m/z=44$ - Polypropylene glycol (PPG) delta $m/z=58$
Glassware - bottles, vials, measuring cylinders, funnels, etc	- Improper cleaning of glass - Non-borosilicate glass (glass that is not Pyrex or equivalent)	- Detergents from cleaning (PEG series, delta $m/z=44$) - Adduct ions, e.g., sodium (Na^+, $m/z=23$), potassium (K^+, $m/z=39$) - Siloxanes
Silica capillary tubing	nanoACQUITY plumbing and columns	Siloxanes

Table 8: Common sources of contamination

Potential sources	Examples	Possible contaminants
Plasticware and tubing	Bottles, vials, well plates, measuring cylinders, funnels, storage carboys, transfer tubing	• Phthalates • Plasticizers, e.g., eurcamide • Slip agents, e.g., stearate
Dishwashing	• Cross contamination from dishwashers • Non-HPLC grade water • Detergents	Detergents from cleaning (PEG series delta m/z=44)
Other cleaning procedures	Waters TUV/PDA detector cleaning	Phosphate ions (m/z=98)
Sample matrix	Biological samples	Proteins, lipids (fats), carbohydrates, salts (sodium, potassium, chloride, etc.)
Sample preparation	Triton X-100, Triton X-114, Brij35, guanidine HCl, urea, etc.	Detergents (PEG series delta m/z=44) and chaotropic agents used to solubilize tissues to extract analytes
Instrument parts & components	Manufacturing residues, cleaning agents, metal ions	Detergents, hydrocarbons, adduct ions
Columns	Silica packing material (especially non BEH or Xbridge column)	• Siloxanes • Ligand chains