Controlling Contamination in UltraPerformance LC®/MS and HPLC/MS Systems

715001307, Rev. F

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Table of Contents

1 Preventing Contamination

To minimize contamination in LC/MS, follow good laboratory practices:

Select, prepare, and handle solvents correctly

Close attention to the selection and use of solvents (or mobile phases) is a critical safeguard against contamination. Waters recommends the following procedures when using solvents.

NOTE: *The recommendations that follow are based on experiences in Waters' labs. Any specific brands mentioned are supplied as a guide only. Waters did not receive compensation for any recommendations.*

1.1 Use clean, particle-free solvents

When preparing mobile phase, always use chemically clean and particle-free solvents and reagents. Solvents must be prefiltered by the manufacturer with a 0.2-µm (or smaller) filter. Based on current analysis, Waters recommends using the following solvent brands (or their equivalents):

- J.T.Baker®: LC/MS Grade
- Burdick & Jackson: B&J Brand® LC-MS Grade
- Fisher: Optima® LC/MS Grade

NOTE: *If using solvent brands other than the ones listed above, check with the manufacturer to verify that the solvents were prefiltered with a 0.2-µm (or smaller) filter.*

CAUTION: ***DO NOT PERFORM FURTHER FILTRATION ON PREFILTERED SOLVENTS; ADDITIONAL FILTERING CAN INTRODUCE CONTAMINATION.***

1.2 Use ultrapure water

Use ultrapure (i.e., particle-free, chemically clean, 18-megaohm cm resistivity) water. This will reduce the amount of impurities in the water that can collect on the column during equilibration with the weak solvent.

Ultrapure water is water that has been purified through a system that targets contaminants detrimental to UPLC®/MS and HPLC/MS systems. The purification process should include *all* of the following steps:

a. Reverse osmosis (to remove most contaminants)
b. UV sterilization (to kill bacteria
c. Ion exchange (to remove any remaining ions)
d. Carbon filtration (to remove any remaining organics)
e. A pharmaceutical-grade 0.2-µm membrane filter (to remove any remaining particulates)

CAUTION: ***IF USING A PURIFICATION SYSTEM, YOU MUST PERFORM REGULAR MAINTENANCE ON IT.***

If outlet lines have been without flow for more than 24 hours, flush them for 20 minutes to eliminate bacterial growth.

Once the water has been purified, do not store it for longer than 24 hours without taking measure to prevent the growth of microorganisms.

NOTE: *If using bottled water, pay attention to the expiration date suggested by the manufacturer, and discard the water after that date. Open bottles of water can become contaminated.*

1.3 Prevent microbial growth

Aqueous mobile phases and water are susceptible to microbial growth, which can cause peaks to appear during gradient operation and increase background absorbance during isocratic operation. Microbial growth can also block filters, frits, and columns, and can cause check valves to malfunction. Such problems can cause high column or pump backpressure and ultimately lead to premature column failure and system shutdown.

To prevent microbial growth in mobile phase, prepare, filter, and degas aqueous mobile phase daily. During shutdown or over a long period of time (such as a weekend), flush the system completely with water, followed by 10% (minimum) of an appropriate organic solvent (such as acetonitrile or methanol).

CAUTION: ***DO NOT STORE THE SYSTEM IN WATER OR >90% AQUEOUS MOBILE PHASE. MICROBIAL CONTAMINATION MAY RESULT.***

1.4 Degas all solvents

Degas all mobile phase before using it. Degassing can help ensure a stable baseline and consistent analytical results.

NOTE: *If your system contains an inline degasser, do not perform further degassing.*

1.5 Minimize the use of additives

a. To reduce background, use the lowest concentration of mobile phase additive (e.g., 0.1% formic acid, not 1%) that is compatible with the chromatography.

b. Use the highest quality of additives available.

c. Use additives (for example, formic acid) that have low concentrations of iron and other metal ions. Acetic acid can contain a significant amount of iron and other metal ions.[1]

d. To prevent precipitation, avoid using inorganic salts or additives in high organic eluents. Such salts or additives can precipitate at the high-organic end of the gradient.

e. Use additives that are volatile and compatible with mass spectrometers.

CAUTION: ***IF YOU ARE USING A MASS SPECTROMETER, AVOID USING NON-VOLATILE ADDITIVES SUCH AS SODIUM (Na^+), POTASSIUM (K^+), OR PHOSPHATE (PO_4^3).***

CAUTION: ***SOME ADDITIVES CAN BE INCOMPATIBLE WITH MASS SPECTROMETERS. CONSULT THE DOCUMENTATION SHIPPED WITH YOUR SYSTEM FOR COMPATIBLE ADDITIVES.***

f. Additives containing ammonium (NH_4^+), acetate, formate, or carbonate are recommended.

CAUTION: ***SOLVENTS WITH A PH GREATER THAN 10 DISSOLVE SILICA. IF YOUR SYSTEM CONTAINS FUSED SILICA AND GLASS COMPONENTS (FOR EXAMPLE, IF YOU HAVE A NANOACQUITY UPLC™ SYSTEM), AVOID USING SOLVENTS WITH A PH GREATER THAN 10.***

1. Stuart Williams, "Ghost peaks in reversed-phase gradient HPLC: a review and update," *Journal of Chromatorgraphy A,* 2004, 1052, 1-11.

g. To flush the system after using mobile phase containing additives, run a wet prime with at least five system volumes of water, followed by 10% (minimum) of an appropriate organic solvent (such as acetonitrile or methanol).

1.6 Use miscible solvents

Make sure solvents are miscible. Be aware that proteins (from tissues, blood, or serum samples) may precipitate in high (>40%) organic solvents. The precipitated proteins can clog injectors and tubing, or adsorb the analyte or contaminants.

1.7 Store solvents in clean glass reservoirs with covers

a. Store the solvent in a covered reservoir to prevent airborne contaminants from entering the solvent:

b. Store mobile phases in borosilicate glass reservoirs. Borosilicate glass should be type 1, class A[2] or type 3.3[3].

Store aqueous mobile phases in amber or brown-stained borosilicate glass reservoirs to retard growth of microorganisms (e.g., algae).

Never store liquids in plastic, which may contain plasticizers and thus promote organic contamination.

NOTE: *The brown bottles in which the manufacturer ships solvents are* ***not*** *borosilicate and should not be used to store aqueous solutions.*

c. Use aluminum foil to cover the reservoirs.

CAUTION: ***DO NOT USE PARAFILM® OR OTHER PLASTIC FILMS TO COVER SOLVENT RESERVOIRS.***

d. Use the smallest solvent reservoir appropriate for your analysis (it will depend on your flow rate and the length of your runs).

e. Do not top off solvents. Instead, discard old solvent, rinse the bottles and solvent inlet filters with the solvent that will be used, and then refill with fresh solvent. Finally, do a wet prime.

1.8 Clean laboratory glassware properly

a. Any glass container used to prepare or store mobile phase must be thoroughly cleaned before use. To clean laboratory glassware:

- First, rinse it with organic solvent and then water.
- Next, rinse it with the solvent that will be put into it.
- If more aggressive cleaning is required (for example, when the container's history is unknown), use the following procedure: Sonicate with 10% formic or nitric acid, then water, then methanol or acetonitrile, then water. Repeat two more times.

b. Wash glassware separately from other containers.

CAUTION: ***DO NOT WASH GLASS BOTTLES IN DETERGENT, WITH OTHER GLASSWARE, OR IN WASHING FACILITIES THAT MAY HAVE DETERGENT RESIDUE. WASHING GLASSWARE IN A COMMON DISHWASHING FACILITY CAN CONTAMINATE IT WITH DETERGENT RESIDUES, WHICH MAY CONTAIN POLYETHYLENE GLYCOL (PEG) AND OTHER "STICKY" SUBSTANCES. VINYL-COATED STEEL RACKS CAN BE AN ADDITIONAL SOURCE OF CONTAMINATION.***

c. Store glassware used to prepare or store mobile phase separately from other common-use glassware.

2. ASTM International, "Standard Specification for Glasses in Laboratory Apparatus," E 438 - 92.
3. ISO 3585: 1998 E, "Borosilicate glass 3.3 - properties," 1998.

d. If glassware or solvent reservoirs become contaminated with microbial growth, treat them in an autoclave. Remove and replace all filters and tubing between the mobile phase reservoir and the instrument. Finally, purge the system with acetonitrile or methanol and let it sit overnight.

Prepare and handle samples correctly

Make sure your samples are particle-free. At the same time, you must take care not to introduce contaminants during the process of preparing and handling samples.

1.9 Use efficient cold traps

Use an efficient cold trap when concentrating, lyophilizing, or distilling your sample. Otherwise, vacuum pump oil can backstream and cause contamination.

1.10 Use clean vials, caps, and plates

a. Use Waters-brand vials; they have been certified as contaminant-free.[4] Other vials may not be clean or may have caps containing adhesives that can contaminate the sample manager.

b. Make sure the liner on your vial and bottle caps does not contain contaminants (check the manufacturer's description):

- Do *not* use vial or bottle caps lined with paper. Paper can be a source of contaminants.
- Single-layered septa are acceptable if they do not contain plastics or adhesives that can contaminate the sample manager (PTFE is recommended).

c. Use Waters-brand wellplates. Be aware that other brands may leach plasticizers (e.g., diisooctylphthalates).

d. Foil-lined plate covers are acceptable as long as the aluminum does not touch the solvent (thereby causing a possible reaction).

Use clean fittings and tubing

1.11 Use clean, inert materials for connections

a. Connections that come into contact with solvents or sample include stoppers, O-rings, check valves, and solvent inlet filters (sinkers).

b. Be aware that tubing made of polymers (such as polyvinylchloride, or PVC) may contain plasticizers or other contaminants.

Wear gloves

1.12 Wear particulate-free, powder-free, non-latex gloves

Use Waters' sterile nitrile gloves (see Table 1) when:

- Handling parts of the UPLC or HPLC system that come into contact with mobile phase or sample
- Replacing old parts with parts that have the label "Critical Clean"

NOTE: *To avoid the risk of incidental skin contact, do not wear finger cots as a substitute for gloves.*

4. *Waters® LC/MS Certified Sample Vials*, 720001517EN, Waters Corporation, Milford, MA, 2006.

Table 1: Nitrile Gloves

Part Number	Description	Qty
700002964	Sterile Nitrile Gloves, Size 7	3 pairs
700002965	Sterile Nitrile Gloves, Size 9	3 pairs

Open the gloves by unfolding the packaging leaves until the cuffs are exposed (Figure 1).

Figure 1 - Removing Gloves from Package

Grasp the cuff of one glove and pull it over your hand, leaving the cuff turned up. Repeat with the other glove. Then turn down the cuffs of both gloves.

CAUTION: ***WHEN PUTTING ON GLOVES, DO NOT TOUCH THE GLOVE FINGERS WITH YOUR BARE HAND. ONCE THE GLOVES ARE ON, DO NOT TOUCH ANYTHING OTHER THAN THE CRITICAL-CLEAN PARTS BEING HANDLED OR SERVICED.***

Use clean columns

1.13 Use clean columns

A UPLC or HPLC column can trap particles, precipitated proteins, and other organic contaminants at the head of the column. These contaminants can adversely affect column lifetime by increasing operating pressure or by altering chromatographic selectivity. In addition, they can slowly bleed off, increasing the background noise.

The column can also adsorb impurities from the solvents. This can occur when you:

- Equilibrate a reversed-phase column (e.g., C18) for long periods of time in high-aqueous conditions
- Run a reversed-phase column isocratically at lower organic concentrations

The adsorbed compounds may elute as distinct peaks or as a smear across the chromatogram. The trace-enriching effect amplifies the amount of contamination present in the solvents or the UPLC or HPLC system.

1.14 Cleaning columns

To clean a contaminated column, wash the column with solvents that will remove the contaminants and not damage the column. Clean the column periodically with high-organic solvent such as 100% acetonitrile. For full instructions on cleaning a column, refer to the care and use instructions provided by the column manufacturer.

WARNING: ***SOME SILICA PACKING MATERIALS WILL DISSOLVE AT PH > 8 IF WASHED IN SOLVENTS CONTAINING ADDITIVES SUCH AS AMMONIUM HYDROXIDE. IF YOUR MOBILE PHASE PH IS GREATER THAN 8, USE A COLUMN THAT IS MORE STABLE TO HIGH PH, SUCH AS THE WATERS ACQUITY UPLC™ BEH OR XBRIDGE™ COLUMN.***

WARNING: ***FOR DETAILED GUIDELINES ON WASHING SILICA PACKING MATERIALS, REFER TO THE CARE AND USE INSTRUCTIONS PROVIDED BY THE COLUMN MANUFACTURER.***

1.15 Storing columns

Store columns in the solvent they originally came with (e.g., 100% acetonitrile). For detailed instructions, refer to the solvent recommendations provided by the column manufacturer.

Check laboratory air

1.16 Take precautions to keep laboratory air clean

Be aware that compounds present in laboratory air can contaminate the LC/MS system:

- Siloxanes are often present in laboratory air.[5] These compounds, which exist in deodorant and other cosmetic products, can cause contamination under certain conditions, such as nanoflow (Figure 2).

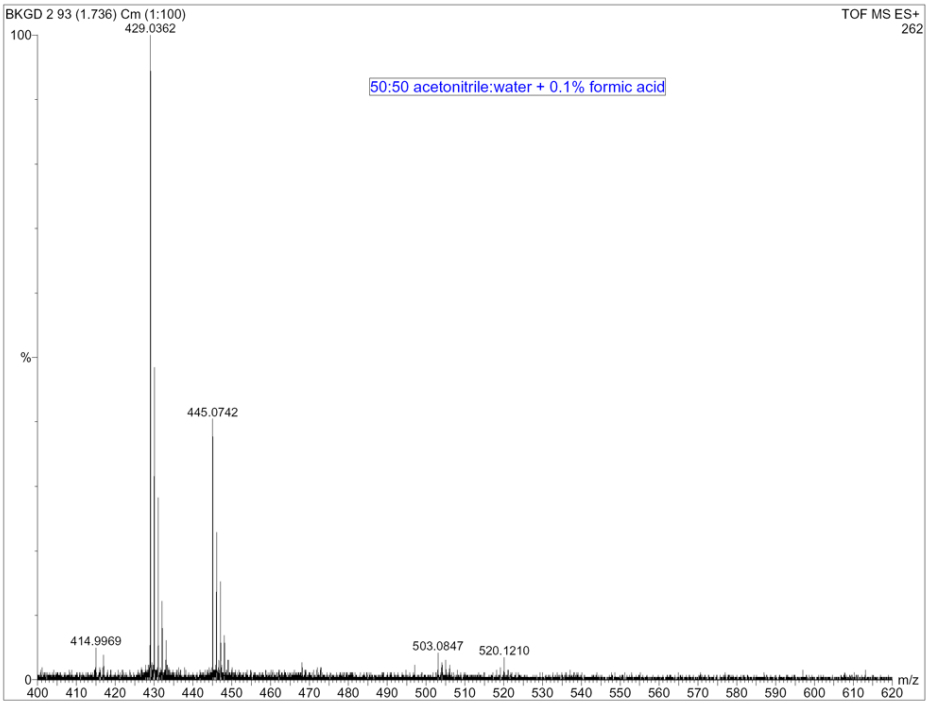

Figure 2 - ESI+ Spectrum Showing Siloxane Contamination

- Phthalates are also omnipresent.[6] Airborne phthalates come from air conditioning filters and can contaminate any solvents or solids that come into contact with the air.

5. Andreas Schlosser and Rudolf Volkmer-Engert, "Volatile polydimethylcyclosiloxanes in the ambient laboratory air identified as source of extreme background signals in nanoelectrospray mass spectrometry," *J. Mass Spectrom.*, 2003; 38: 523-525.
6. Manfred Ende and Gerhard Spiteller, "Contaminants in mass spectrometry," *Mass Spectrometry Reviews,* 1982, 1, 29-62.

2 Troubleshooting Contamination

Even the best efforts at prevention may not completely eliminate contamination. This section outlines a common-sense approach to troubleshooting and cleaning contamination in an LC/MS system. The following tests systematically isolate the problem to either the sample itself or one of the hardware components in the LC or MS system.

Narrow down the problem

2.1 Isolate the problem to the LC or MS system

a. Flush the ESI probe with clean solvent other than mobile phase and connect a syringe infusion kit directly to the ESI probe.

b. Infuse into the MS the mobile phase that you are using in the system (for example, a 50:50 A/B mixture at 0.3 mL/min), making sure to bypass the entire HPLC and solvent management system.

CAUTION: ***DO NOT INFUSE WITH 100% ORGANIC MOBILE PHASE. ANALYTES MAY NOT IONIZE WELL UNDER ELECTROSPRAY CONDITIONS.***

- If contamination levels decrease, contaminants are probably located primarily in the LC system. *Go to the next section, "Troubleshoot the LC System".*
- If contamination spectra do not decrease in intensity, the source of contamination is probably in the MS system. *Proceed to "Troubleshoot the MS System" (page 10).*

Troubleshoot the LC system

2.2 Remove the column

Be sure to remove the column when performing LC troubleshooting. You can replace the column with a union.

NOTE: *Be aware that contaminants can collect and concentrate on a column (trace enrichment) when you run low-concentration organic mobile phases (e.g., initial conditions) for a long period of time. The contaminants will elute from the column when a gradient is run. If contamination appears after you troubleshoot the LC system and then reinstall the column, repeat steps 2.4 through 2.6g (but omit the infusion steps) with the column installed.*

2.3 Check the mobile phase

Mix 1 mL of mobile phase A with 1 mL of mobile phase B in a clean vial. Infuse the mixture into the mass spectrometer.

- If contamination exists, the problem is in the bottles or mobile phase. See the guidelines on using clean solvents and containers in Chapter 1, "Preventing Contamination".
- If there is no contamination on infusion, pump the 50:50 A/B mixture through a clean probe into the mass spectrometer. If you see contamination, it is located in the solvent manager/chromatographic pump or sample manager/autosampler (or both). To determine which component is the source, *go to step 2.4.*

2.4 Make a zero-volume injection

If you see contaminants, the problem is in the solvent manager/chromatographic pump. *Go to step 2.5.* If no contaminants are present, the problem is in the sample manager/autosampler. *Skip to step 2.6.*

2.5 Check the solvent manager/chromatographic pump

Disconnect the solvent manager/chromatographic pump from the sample manager/autosampler and pump directly into the mass spectrometer. If contamination exists, the solvent manager/chromatographic pump is the source of the contamination. Reconnect the sample manager/autosampler and follow the guidelines in sections 3.1 through 3.3. If contamination does not exist, the problem is in the sample manager/autosampler. *Go to step 2.6.*

2.6 Check the sample manager/autosampler

Pump a wash solution (Table 2) through the sample manager/autosampler to waste. Use the same solution to flush the needle wash flow path. Also inject large volumes (e.g., full loop with 3X overfills) of the cleaning solution. Then return to mobile phase and flush thoroughly. If contamination exists, determine whether it is in the sample, the diluent, the infusion device, or the sample container.

a. *Check the solvent, water, and acid used for dilution.* Infuse the sample diluent — for example, a mixture of equal parts water and either acetonitrile or methanol plus 0.1% formic acid — into the mass spectrometer to check for contamination. If there is no contamination in this "blank", then the contamination came with the original sample. If the contamination persists, *go to step b.*

b. *Check the infusion device and sample container.* Clean or replace each component. Then repeat the infusion test. If the infusion device and container are clean, *go to step d*.

c. *Change the injection size.* If replacing the sample diluent did not solve the problem, try adjusting the injection volume by a factor of 2 or more. If the contamination increases or decreases in proportion to the injection volume change, it is probable that the sample is contaminated. New sample or further sample clean-up may be required. If the sample volume change has no effect on the size of the carryover peak, *go to Chapter 3, "Cleaning to Eliminate Contamination".*

d. *Check the needle wash solutions.* Are they the appropriate wash solvents? If not, use the correct wash solutions. Make another injection and check for contamination. If it still exists, *go to step e*.

e. *Check the tubing and fittings on the injector, especially the injector outlet to column inlet.* If there is dead volume, contamination can accumulate in those spaces. Replace the tubing and fittings. Make another injection and check for contamination. If it still exists, *go to step f*.

f. *Replace the needle.* Then make another injection. If contamination persists, *go to the step g*.

g. *Replace the other injector parts (e.g., needle wash port, injector valve pod).* Refer to the operator's manual for specific injector parts that can be replaced. Flush the system with mobile phase. Make another injection and then check for contamination.

h. *If contamination still exists,* skip to the next section, "Troubleshoot the MS System."

i. *If contamination does not exist*, go to step 2.7.

2.7 Reinstall the column

Reinstall the column and then check for contamination. If contamination appears, repeat steps 2.4 through 2.6g with the column installed (but omit the infusion steps).

NOTE: *When troubleshooting LC systems with a column installed, you do not need to repeat steps 2.6a and 2.6b.*

Troubleshoot 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.*

2.8 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)

2.9 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.

Clean or replace the contaminated component

2.10 Clean or replace the last added component

If background noise is high after any test, clean or replace the last component added. For instructions on cleaning LC/MS systems, see Chapter 3, "Cleaning to Eliminate Contamination".

3 Cleaning to Eliminate Contamination

If you need to clean the LC/MS system, an understanding of contaminants and their sources is essential. For information, *see* Major Contaminants and Their Sources, page 16.

CAUTION: ***DO NOT ATTEMPT TO CLEAN THE SYSTEM UNTIL YOU HAVE FOUND AND ELIMINATED THE SOURCE OF CONTAMINATION.***

NOTE: *These cleaning guidelines:*

- *are based on traditional techniques using materials that are readily available in the laboratory*
- *apply primarily to reversed-phase LC/MS*

Cleaning LC Systems

3.1 General guidelines

To clean contamination in LC systems, use the highest-purity solvent mixtures (see section 1.1). If you know what the contaminant is, use the mixture in which it is most soluble. *See Table 2 on the next page* 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 *(see Section 1.2)* 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 (e.g., Waters' ACQUITY UPLC System Operator's Guide (PN 71500082502) or AutoPurification Operator's Guide (PN 71500135202).*

Table 2: Recommended Cleaning Mixtures for LC

	LC MIXTURE 1 [A]	LC MIXTURE 2[A]	LC MIXTURE 3 [A]	LC MIXTURE 4 [A]
PURPOSE	General purpose solution for nanoACQUITY or other applications where use of high-pH mobile phase is not advisable	"Universal" wash solution for high background spectra	Use to remove PEG and amide contamination	Strong acid wash
CAUTION			• ***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.***	• ***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 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.***
MIXTURE	• 100% 2-propanol (isopropyl alcohol, or IPA)	• 25% acetonitrile • 25% methanol • 25% 2-propanol • 25% water • 0.2% formic acid	• 50% acetonitrile • 49% water • 1% ammonium hydroxide	• 30% phosphoric acid • 70% water

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

3.2 Cleaning the solvent manager/chromatographic pump

Pump the cleaning solution through the solvent manager/chromatographic pump (or solvent manager/chromatographic pump and sample manager/autosampler) to waste. Then flush thoroughly with mobile phases.

3.3 Cleaning the sample manager/autosampler

Pump the cleaning solution through the sample manager/autosampler to waste. Use the same solution to flush the needle wash flow path. Also inject large volumes (full loop) of the cleaning solution. Then return to the mobile phase and wash solutions required for analysis and flush thoroughly.

3.4 Cleaning the column

See Section 1.14, "Cleaning Columns".

Cleaning MS Systems

3.5 Remove and clean or replace suspected MS components

Remove and clean or replace MS components suspected of causing contamination. Use the highest-purity solvents. The objective should be to dissolve contamination from the surface of the MS component.To avoid recontaminating clean or new components, use a methodical cleaning process. In some cases you should clean or replace one component at a time, working from upstream to downstream (in terms of solvent flow). In other cases it is better to clean all suspected components simultaneously. Be sure to use ultra-pure solvents and clean glassware (see Chapter 1, "Preventing Contamination").

a. Carefully wipe the component with a clean swab or lint-free wipe.

CAUTION: ***TO AVOID CONTACT WITH TOXIC CONTAMINANTS, BE SURE TO WEAR GLOVES AND EYE PROTECTION.***

b. Sonicate components in solvent for between 15 minutes to 1 hour. See Table 3 for recommended MS cleaning solutions.

Table 3: Recommended Cleaning Mixtures for MS

	MS MIX 1[A]	MS MIX 2 [A]	MS MIX 3 [A]	MS MIX 4 [A]	MS MIX 5 [A]
PURPOSE	Use to remove hydrophilic contaminants.	Use to remove hydrophobic contaminants.	Use to remove most contaminants.	For metal components only; use to remove most contaminants.	For metal components only; use when contamination comes from hydrocarbons, oil, or grease. Follow with sonication in methanol.
CAUTION				• ***DO NOT SONICATE PEEK COMPONENTS OR T-WAVE ASSEMBLIES IN MIXTURE 4.*** • ***AFTER SONICATING WITH MIXTURE 4, BE SURE TO SONICATE IN MIXTURE 3.***	• ***DO NOT SONICATE PEEK COMPONENTS OR T-WAVE ASSEMBLIES IN MIXTURE 5.*** • ***BE SURE TO FOLLOW THE CORRECT SOLVENT SEQUENCE WHEN WASHING WITH MIXTURE 5 OR OTHER SOLVENTS THAT ARE IMMISCIBLE WITH WATER.***
MIXTURE	• Water	• Organic solvent such as methanol, acetonitrile, or 2-propanol	• 50% organic solvent • 50% water	• 45% acetonitrile or methanol • 45% water • 10% formic acid	• Chlorinated solvents, hexane, or acetone

A REFER TO YOUR DETECTOR'S OPERATOR'S MANUAL FOR SPECIFIC ADVICE ON CLEANING MS COMPONENTS.

NOTE: *Effective removal of contamination may require several sonication steps, using fresh cleaning solvent at each step.*

c. If these solutions fail to reduce contamination levels, sonicate components in a sequence of solvents as follows:

- *Dichloromethane*
- Acetone
- 2-propanol

Each sonication step should last between 15 minutes to 1 hour, depending on the degree of contamination and the power output of the sonication equipment.

CAUTION: ***DO NOT SONICATE PEEK COMPONENTS OR T-WAVE™ ASSEMBLIES IN CHLORINATED SOLVENTS, HEXANE, ACETONE, OR ACIDS AS SOLVENTS. DOING SO COULD DAMAGE THE COMPONENTS OR ASSEMBLIES.***

d. Be sure to rinse the glassware thoroughly and use fresh, clean solvent between each step.

e. After final sonication, remove the MS component from the cleaning solution. Quickly dry the component with a strong stream of clean, dry nitrogen.

CAUTION: ***QUICK, THOROUGH DRYING IS NECESSARY TO PREVENT SOLVENT SPOTS, WHICH CAN AFFECT FUTURE MS PERFORMANCE***

3.6 Front panel injector

After decontaminating the rest of the plumbing, strip and clean the front panel injector according to manufacturer instructions. Pay particular attention to the rotor seal, on which mechanical wear (observable as circular grooves) can serve as a site of contamination. Replace the seal if necessary.

3.7 API source

Because it can be exposed to a large quantity of sample material during normal operation, the atmospheric-pressure ionization (API) source is the most common location of MS contamination. Disassemble and clean the source using normal maintenance procedures. Sonicate API source components in solvent for between 15 minutes to 1 hour.

CAUTION: ***FOLLOW THE GUIDELINES FOR SOLVENT QUALITY AND GLASSWARE CLEANLINESS.***

NOTE: *Ion optics from the ion transfer region forward are an unlikely location of contamination detectable by MS analyses.*

3.8 API probes

Clean API probes by pumping cleaning solvent through them into a clean waste container. Replace the following subassemblies:

- APCI (atmospheric-pressure chemical ionization) and ESI (electrospray ionization) probe capillaries
- APCI filter
- APCI heater
- ESI probe tip
- LC union at the rear of the ESI probe

3.9 LC tubing

Replace contaminated LC tubing rather than cleaning it through repeated flushing.

3.10 Nitrogen gas tubing

If nitrogen tubing has become contaminated through solvents or a source flood, it may be necessary to replace all affected tubing and fittings in one step.

3.11 Nitrogen gas supply

If the nitrogen gas generator or its filters are near the end of their lifetime, they may be a source of contamination. To check for generator contamination, switch temporarily to a clean cylinder. If the contamination disappears, replace the gas generator cartridge.

4 Major Contaminants and Their Sources

This section lists some major contaminants in LC/MS systems, along with their sources and spectra.

NOTE: *For more information on major contaminants, see PEG master list and Background Ion Master List.*

4.1 Polyethylene glycol (PEG) or PEG-like materials

PEG is a synthetic polymer produced in a range of molecular weights. Common sources of PEG contamination include:

a. Organic solvents
   - Methanol
   - 2-propanol
   - Acetonitrile
   - Water

b. Mass spectrometer calibration solution
c. Hand cream
d. Detergent
   - Triton X-100, etc.
   - Glassware detergents

e. Cutting solutions in machining
f. Column manufacturing

Figure 3 shows a typical PEG spectrum, exhibiting a series of mass peaks separated by 44 Da.

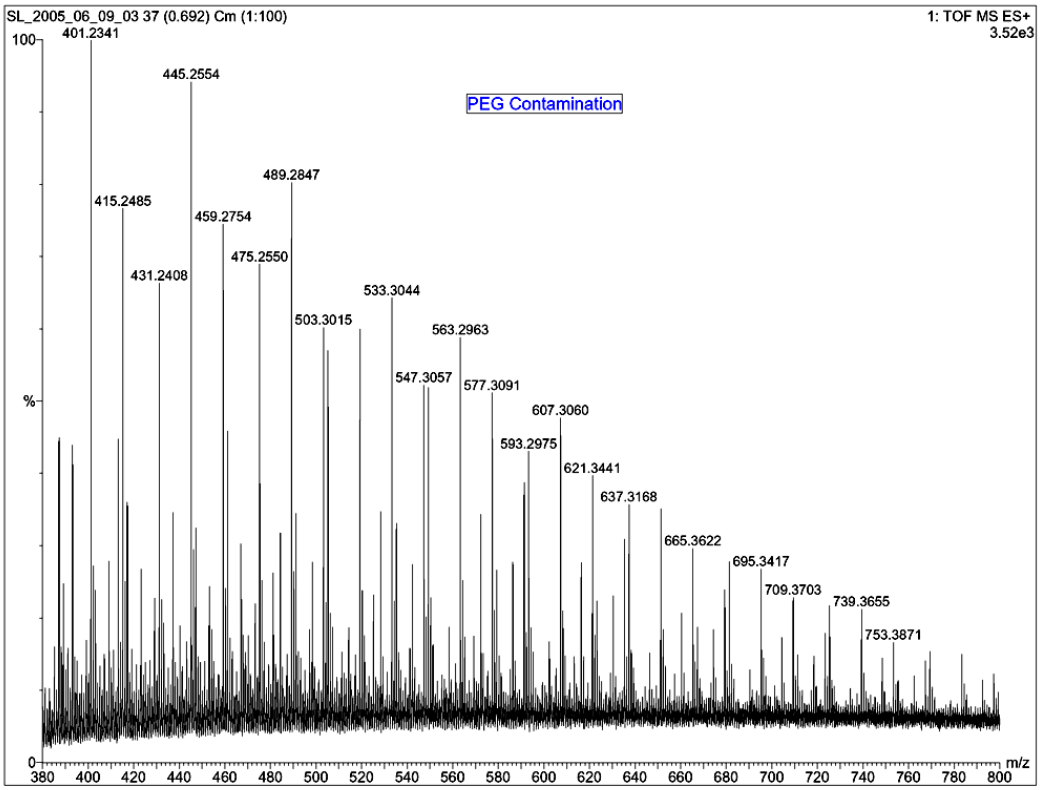

Figure 3 - PEG Contamination Spectra

4.2 Metal ions

Metal ions such as lithium (Li), sodium (Na), potassium (K), copper (Cu), platinum (Pt), and iron (Fe) can be sources of contamination.

For example, iron forms adducts with varying numbers of acetate in acetic acid or acetate mobile phases. Iron can contaminate an LC/MS system through the following sources:

- Solvents such as water and acetonitrile
- Acetic acid (lower in formic acid)
- Formic acid
- Non-passivated stainless steel parts
- Titanium or inert metal parts fabricated with steel tools

Figure 4 shows the typical pattern of Fe-acetate cluster spectra. The strongest ion (base peak intensity, or BPI) mass may be different, depending on the number of acetates in the cluster. The upper spectra are based on the MassLynx isotope model.

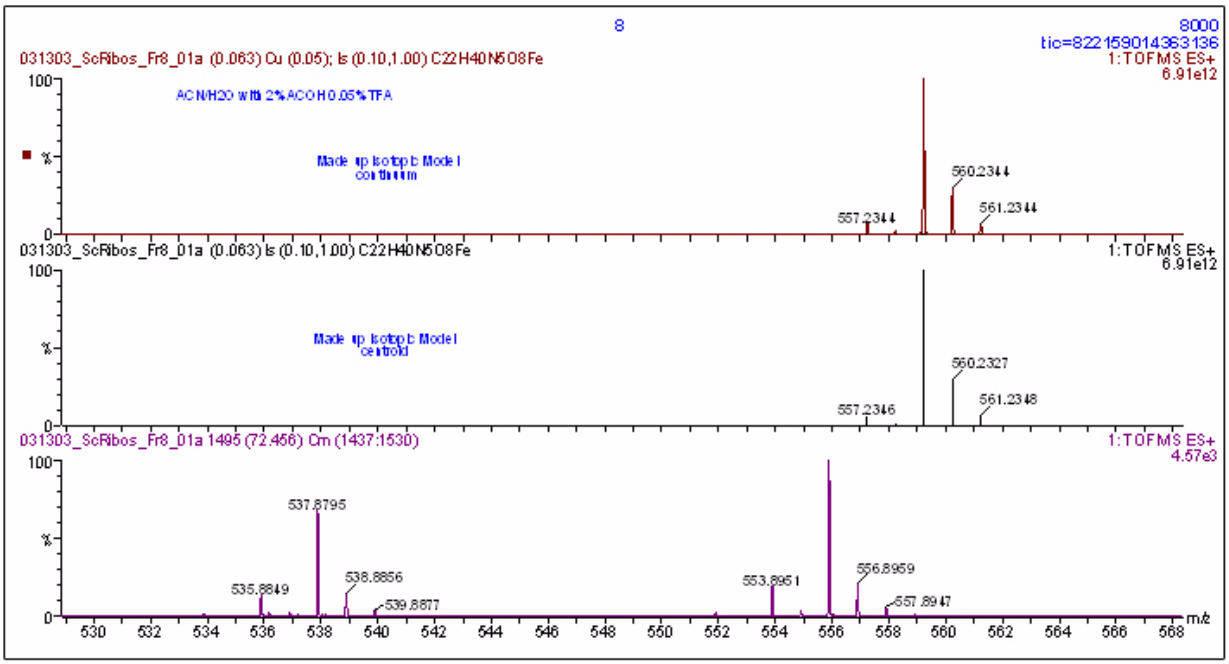

Figure 4 - Fe Contamination Spectra

4.3 Phthalates

Phthalates are chemical compounds used chiefly as plasticizers, and can cause contamination. The compounds can be detected on a wide range of laboratory materials, including water and other solvents, laboratory air, and plastic materials such as tubing and water storage containers. Common phthalates include di-2-ethyl hexyl phthalate (DEHP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), and diisooctyl phthalate (DIOP).

Diisooctylphthalates can form the following adducts:

- $[M+H]^+$ = 391
- $[M+Na]^+$ = 413
- $[M+K]^+$= 429
- $[2M+NH_4]^+$= 798
- $[2M+Na]^+$ = 803

4.4 Slip agents (amides)

Avoid using components packed in plastic bags containing slip agents, or amides. The three most commonly used amides are:

- Oleamide ($[M+H]^+$=282)
- Stearamide ($[M+H]^+$=284)
- Erucamide ($[M+H]^+$=338)

Figure 5 shows a spectrum revealing amide contamination.

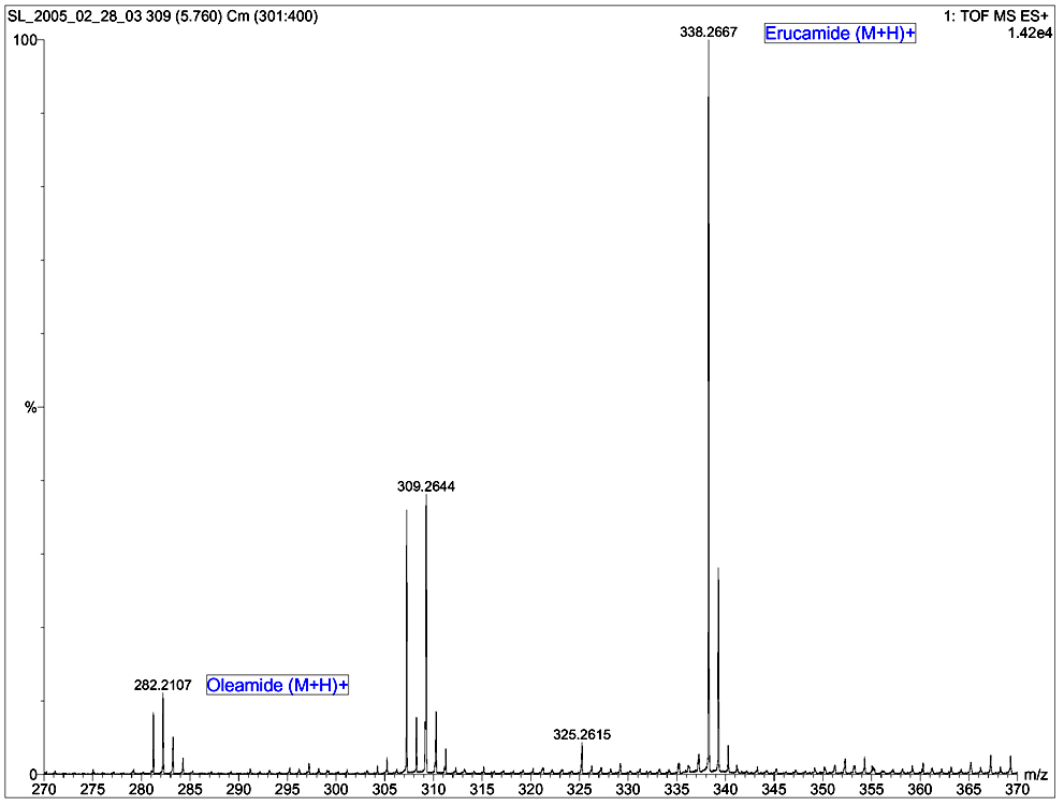

Figure 5 - Contamination Spectrum of Oleamide and Erucamide in Zip Bag