Controlling Contamination in LC/MS Systems

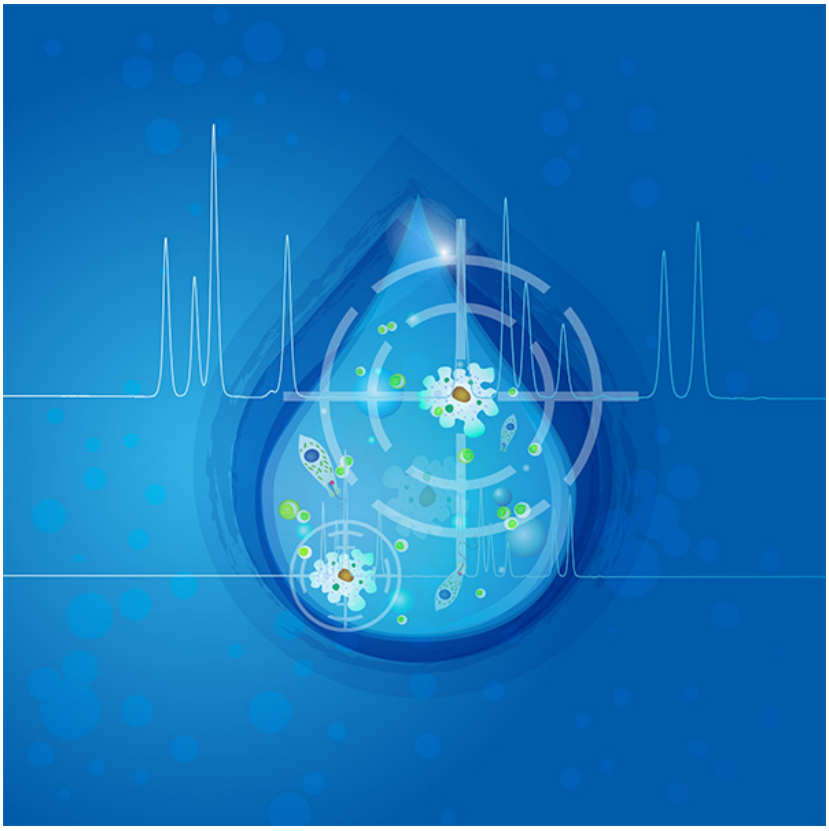
Best Practices

This document outlines best practices for controlling contamination in UPLC/MS and HPLC/MS systems (both clinical and non-clinical). A good contamination control plan includes measures for preventing, troubleshooting, and cleaning contamination.

Important: This document updates, supersedes, and supplements all other Waters documents on preventing, troubleshooting, and cleaning contamination.

Note: To understand what causes contamination, see Major contaminants and their sources, page 31.

Note: If you have followed the recommendations in this document and still need help with contamination, contact your Waters representative.



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Preventing contamination

Prevention is the most important factor in controlling contamination. It is much easier to prevent contamination than it is to troubleshoot or eliminate it. To prevent contamination, follow the steps in this section.

Select, prepare, and handle solvents correctly

Close attention to the selection and use of solvents (or mobile phases) is a critical safeguard against contamination (see Possible effects of low-quality solvents, page 23). Waters recommends the following procedures when using solvents.[1]

Note: For more information on solvent recommendations and cautions, see your system user guide.

Use clean, particle-free solvents

Caution: Failure to use the proper grade of solvent for your application causes excessive background noise and loss of sensitivity.

When preparing mobile phase, always use chemically clean and particle-free solvents (Table 3) and reagents. Solvent must be UPLC-grade or a quality level suitable to the application being run. (See Solvent considerations, page 23.)

Use fresh solvents

Use the freshest solvents available; do not store solvents without taking measures to prevent microbial growth (see Store solvents in clean glass reservoirs with covers, page 6). When solvents are exposed to the atmosphere, they become vulnerable to airborne contamination.

Caution: If using organic solvents, pay attention to the expiration date suggested by the manufacturer. Open bottles of solvent can become contaminated.

1. The recommendations are based on experiences in Waters' laboratories.

Use ultra-pure water

Use ultra-pure water (defined as water that has been purified through a system that targets contaminants detrimental to UPLC®/MS and HPLC/MS systems).

Note: See Recommended water purification process, page 29.

Ultra-pure water is sterile and contains no particles greater than 0.2 microns, no detectable ionizable compounds (>18-MOhm.cm resistivity), and low UV absorbance at the monitoring wavelengths. Use of ultra-pure water reduces the number of impurities in the water that can collect on the column during equilibration with the weak solvent.

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 back pressure 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 (such as a weekend), flush the system completely with water. Then flush with 10% (minimum) of an appropriate organic solvent (such as acetonitrile or methanol).

Caution: To prevent microbial contamination, do not store the system in water or >90% aqueous mobile phase.

Minimize the use of additives

Caution: To prevent microbial growth, do not add additives to stock bottles.

- To reduce background, use the lowest concentration of mobile phase additive (for example, acid, base, or buffer that is compatible with good, stable chromatography). The more additive you use, the more contamination can be introduced into the mobile phase.
- When developing a new method or transferring an old one to a new column, eliminate any additives that no longer affect the chromatography. For example, TEA is no longer necessary on many columns to obtain good peak shape for basic compounds.
- Use the highest-quality additives available (at minimum, LC/MS-grade additives)
- 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.
- 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.
- 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.

- Volatile 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 or M-Class UPLC system), avoid using solvents with a pH greater than 10. Avoid storing the system in high pH. Flush and store in methanol or a minimum of 10% organic with no additives or buffers.

- To flush the system after using mobile phase containing additives, flush the system for at least 5 minutes (at least 50 mL of water). Follow with 10% (minimum) of an appropriate organic solvent (such as acetonitrile or methanol).

Use miscible solvents

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

Store solvents in clean glass reservoirs with covers

- Store mobile phases in clean amber or brown-stained borosilicate glass reservoirs (see Clean laboratory glassware properly, page 6). Borosilicate glass must be type 1, class A, or type 3.3.

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

Caution: Never store liquids in plastic, which can contain plasticizers (for example, phthalates) and thus promote organic contamination.

- Cover the reservoir to prevent airborne contaminants from entering the solvent.
- To cover the reservoir, use aluminum foil or caps supplied with the system.

Caution: Do not use Parafilm® or other plastic films to cover solvent reservoirs.

- Use the smallest solvent reservoir appropriate for your analysis (it depends on your flow rate and the length of your runs).
- Do not top off solvents. Instead, discard old solvent, rinse the bottles and solvent inlet filters with the solvent that is used, and then refill with fresh solvent. Finally, prime the system.

Clean laboratory glassware properly

Any glass container used to prepare or store mobile phase must be thoroughly cleaned before use.

Standard cleaning process

Caution: Never wash glassware with detergents or in a dishwasher.

Caution: Always clean glassware with the same mobile phase/solvent you use for the chromatography.

Rinse glassware with organic solvent and then water.

Aggressive cleaning process

If more aggressive cleaning is required (for example, when the container's history is unknown), use the following procedure:

1. Sonicate with 10% formic or nitric acid, then water, then methanol or acetonitrile, then water.
2. Repeat two more times.

3. Store glassware used to prepare or store mobile phase separately from other common-use glassware.
4. If glassware or solvent reservoirs become contaminated with microbial growth, treat them in an autoclave:

   a. Remove and replace all filters and tubing between the mobile phase reservoir and the instrument.
   b. Purge the system with acetonitrile or methanol and let it sit overnight.

Prepare and handle samples correctly

Make sure that 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.

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.

Use clean vials, caps, and plates

1. Use Waters-brand vials; they have been certified for certain levels of cleanliness. (Select the vial appropriate for your application.) Other vials can contain contaminants that interfere with the application and contaminate the system.
2. Make sure the liner on your vial and bottle caps does not contain contaminants (check the manufacturer's description):

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

3. Use Waters-brand wellplates. Other brands can leach plasticizers (for example, diisooctyl phthalates).
4. Foil-lined plate covers are acceptable as long as the aluminum does not touch the solvent (possibly causing a possible reaction).
5. Do not use self-sealing or adhesive cap mats; they can contain contaminants from the adhesives.
6. Do not use cap mats that are not compatible with the solvents used for sample diluents, mobile phase, and wash solvents.

Use clean fittings and tubing

Use clean, inert materials for connections

1. Connections that come into contact with solvents or sample include stoppers, O-rings, check valves, and solvent inlet filters (sinkers).
2. Labels on inlet tubing can contain contaminants. They must not come into contact with solvents or mobile phases.
3. Some types of tubing (such as polyvinyl chloride, or PVC) contain plasticizers or other contaminants.

Caution: Do not touch or bring surfaces into contact with anything containing contaminants.

Wear gloves

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

Use Waters' sterile nitrile gloves (see Table 4) 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".

Caution: To avoid incidental skin contact, do not wear finger cots as a substitute for gloves.

Replace gloves if they become wet

Caution: Use caution when touching surfaces, even when wearing gloves. Keep gloves dry, and replace gloves if they become wet. Wet gloves can introduce contamination. Change gloves frequently.

Putting on gloves

For instructions on putting on gloves to prevent contamination, see How to put on gloves, page 30.

Use clean columns

Important: For detailed instructions on using and caring for columns, refer to the care and use instructions provided by the column manufacturer. (An example is the ACQUITY UPLC BEH Column Care and Use Manual, 715001371.)

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 carryover and background noise.

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

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

The adsorbed compounds can 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.

Note: For guidelines on cleaning columns, see Cleaning columns, page 17.

Storing columns

Store columns in the solvent they originally came with (for example, 100% acetonitrile). Flush columns to remove buffers and additives before storing them.

Note: For detailed instructions, refer to the solvent recommendations provided by the column manufacturer.

Check laboratory air

Keep laboratory air clean

Compounds present in laboratory air can contaminate the LC/MS system:

- Siloxanes are often present in laboratory air. These compounds, which exist in deodorant and other cosmetic products, can cause contamination under certain conditions, such as NanoFlow (Figure 1).

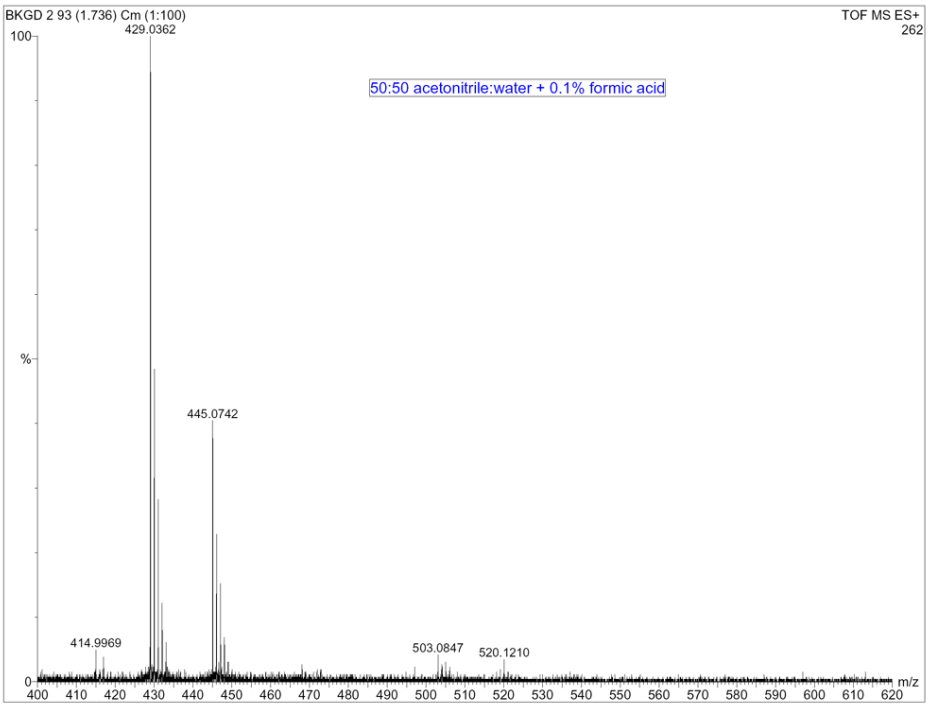
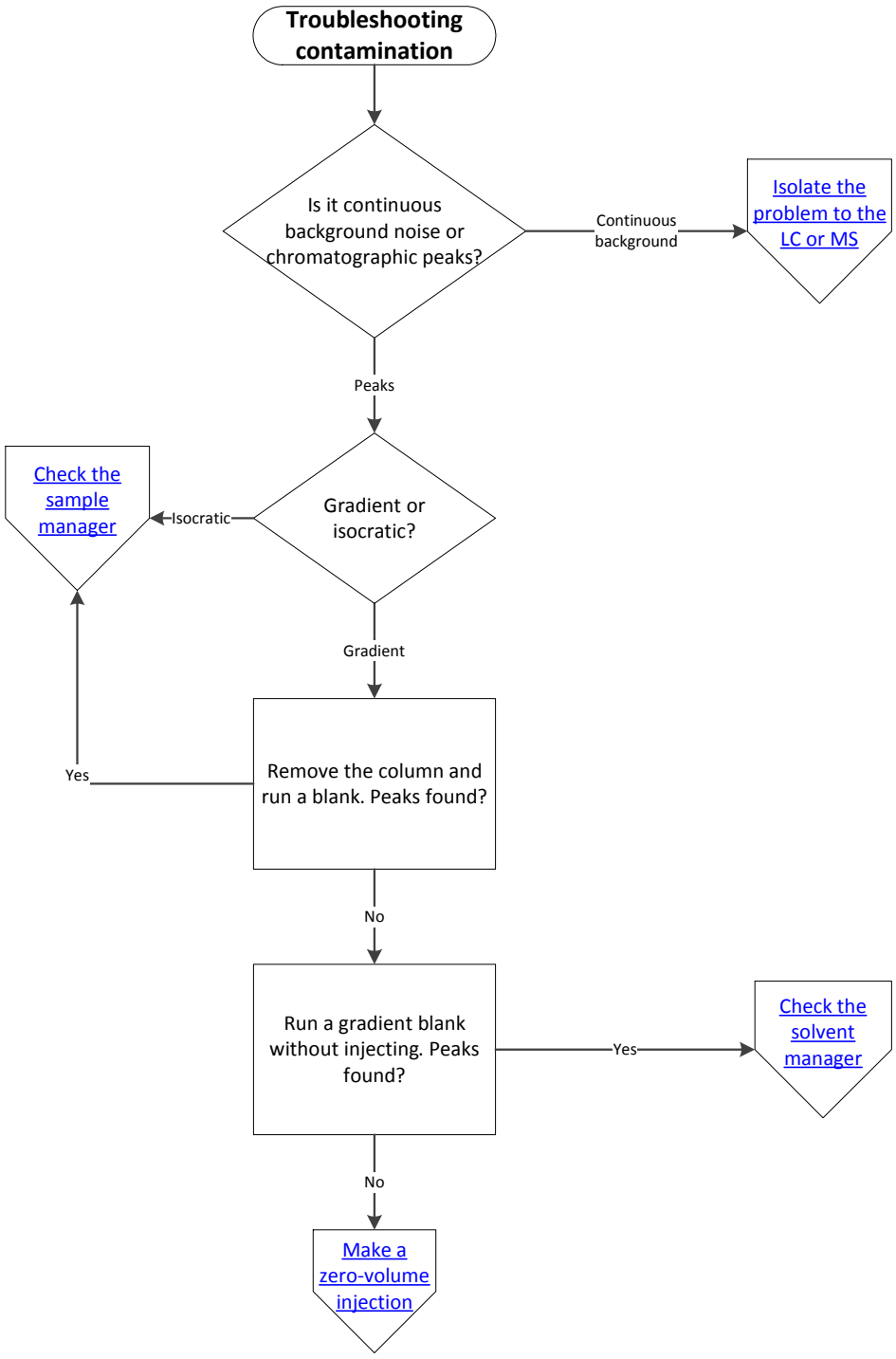


Figure 1 - ESI+ spectrum showing siloxane contamination

- Phthalates are also present in laboratory air. Airborne phthalates come from air conditioning filters and can contaminate any solvents or solids that come into contact with the air.

Troubleshooting Contamination

This section outlines a common-sense approach to troubleshooting contamination in an LC/MS system. Use the flowchart to proceed.



Isolate the problem to the LC or MS system

Note: Complete the flowchart steps on page 11 before beginning this section.

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

Important: 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). Make sure to bypass the entire LC and solvent management system.

- If contamination levels decrease, contaminants are probably located primarily in the LC system. Troubleshoot the LC system, page 12.
- If contamination spectra do not decrease in intensity, the source of contamination is probably in the MS system (source, inlet tubing, fluidics unit, and so on). Troubleshoot the MS system, page 15.

Troubleshoot the LC system

Try a new column

Note: Contaminants can collect and concentrate on a column (trace enrichment) when you run low-concentration organic mobile phases (for example, initial conditions) for a long time. The contaminants elute from the column when a gradient is run.

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 Preventing contamination, page 3.
- 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, Make a zero-volume injection.

Make a zero-volume injection

- If you see contaminants, Check the solvent manager/chromatographic pump.
- If no contaminants are present, Check the sample manager/autosampler.

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 General guidelines, page 16.
- If contamination does not exist, Check the sample manager/autosampler.

Check the sample manager/autosampler

1. *Remove the column if it has not already been removed.*
2. *Pump a wash solution through the sample manager/autosampler to waste.*

   To flush the needle wash flow path, use the same solution you used to flush the pumps. Also inject large volumes (for example, full loop with 3X overfills) of the cleaning solution. If the wash solution is an acid, flush with copious amounts of water. 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.

3. *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, Check the sample.

4. *Check the sample.*

   Infuse a diluted sample into the mass spectrometer.

   Note: PEG can come from detergents used for sample preparation or PEGylated pharmaceutical compounds.

   If the contamination persists, Check the infusion device and sample container.

5. *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, Check the needle wash solutions.

6. *Change the injection volume.*

   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, Change the injection mode.

7. *Change the injection mode.*

   Partial loop needle overfill (PLNO) mode can cause more carryover than partial loop or full loop mode. That is because the sample is pulled through the VDD, which is not flushed with strong needle wash.

   If the contamination persists, Check the needle wash solutions.

8. *Check the needle wash solutions.*

   Are the wash solvents correct? If not, use the correct wash solutions. Make another injection and check for contamination. If it still exists, Check the tubing and fittings on the injector.

9. *Check the tubing and fittings on the injector.*

   In particular, check 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, Replace the needle.

10. *Replace the needle.*

    Replace the needle, then make another injection. Some hydrophobic compounds adsorb to PEEK needles. If contamination persists, try replacing it with a stainless steel needle. If contamination still persists, Replace the other injector parts.

11. *Replace the other injector parts.*

    Replace the other injector parts (for example, 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.

    a. If contamination still exists, Troubleshoot the MS system, page 15.
    b. If contamination does not exist, Reinstall the column.

12. *Reinstall the column.*

    Reinstall the column, and then check for contamination. If contamination appears, repeat the following steps with the column installed (but omit the infusion steps):

    - Make a zero-volume injection, page 12
    - Check the solvent manager/chromatographic pump, page 13
    - Check the sample manager/autosampler, page 13

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.

Important: Do not waste time and resources attempting to remove typical background noise. The sensitive nature of MS systems means that some degree of chemical background is a constant. In addition, different types of MS systems have different degrees of sensitivity. For example, you see a higher background in a more sensitive instrument. Sensitivity is signal-to-noise ratio, not just absolute counts.

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
- LC tubing
- Nitrogen gas tubing
- Nitrogen gas source (for example, 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 possibly have become recontaminated after cleaning. To avoid this problem, clean and replace all suspected parts simultaneously.

Clean or replace the contaminated component

If background noise is high after any test, clean or replace the last component added (see Cleaning to Eliminate Contamination, page 16).

Cleaning to Eliminate Contamination

Warning: To prevent injury, 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 safety data sheets for each solvent and solution in use.

Always use eye protection and gloves when handling solvents or cleaning mixtures.

Caution: Do not attempt to clean the system until you have found and eliminated the source of contamination. If you clean without finding the source, the contamination will soon return and the system will require cleaning again.

Disconnect the LC system from the column and detector before cleaning.

Solvents and acid or base must be of the highest purity for cleaning and use.

If you must clean the LC/MS system, an understanding of contaminants and their sources is essential. For information, see Major contaminants and their sources, page 31.

General guidelines

To clean contamination in Waters systems, use only mobile phase- (highest-) quality solvents and acid or base.[1] Remove the column and detector (any detector, including the mass spectrometer). If you know what the contaminant is, use the mixture in which it is most soluble.

Flush the system component with a high-organic solvent such as 80% organic solvent and 20% water, and then test for contamination. Repeat the procedure until the background is reduced to an acceptable level.

After using any wash, rinse with 50% acetonitrile or mobile phase to remove the cleaning solution. If you use a phosphoric acid wash, pump ultra-pure water (see Use ultra-pure water, page 4) through the system until the pH is close to neutral (pH=7).

1. These cleaning guidelines are based on traditional techniques using materials that are readily available in the laboratory. They apply primarily to reversed-phase LC/MS.

Cleaning columns

Important: If washed in solvents containing additives such as ammonium hydroxideome, silica packing materials dissolve at pH > 8 . If your mobile phase pH exceeds 8, use a column that is more stable to high pH, such as the Waters ACQUITY UPLC™ BEH or XBridge™ column.

For detailed guidelines on washing silica packing materials, refer to the care and use instructions provided by the column manufacturer.

To clean a contaminated column, wash the column with solvents that remove the contaminants and do not damage the column. It is good practice to clean a reversed phase column at the end of a run by flushing it with a high-percentage (for example, 80%) organic mobile phase.

Note: For full instructions on cleaning a column, refer to the care and use instructions provided by the column manufacturer.

Recommended cleaning procedures for MS systems

To clean a standalone MS system, use the cleaning procedure (available in the Waters Support Library) appropriate to your system:

- Mass spectrometry user guides

Recommended cleaning mixtures for LC inlet components

Caution: To prevent cross-contamination, remove the connection to the column and mass spectrometer before cleaning the LC inlet.

To clean the LC inlet, use the recommended cleaning mixtures in Table 1.

For other cleaning procedures that may be appropriate for your system, refer to the Waters Support Library:

- Routine cleaning procedures for separations systems
- UPLC/UHPLC user guides
- Alliance HPLC user guides

Final rinse

After cleaning with any of the mixtures, rinse the LC inlet with one of the following:

- Ultra-pure water (required for phosphoric acid wash)
- 50:50 mixture of water/organic solvent
- 50:50 mixture of the chromatographic method's mobile phases

Important: Always leave the system in the wash solvent that is most similar to your mobile phase (pH) conditions.

Table 1: Recommended cleaning mixtures for LC inlets[1]

Cleaning conditions	Solvent mixtures (v/v)	Stock concentration	Description	Cautions	Comments
1	• 80% 2-isopropanol (IPA) • 10% water • 10% formic acid	• ~99% formic acid	An aggressive cleaning mixture for compounds more soluble in acids	• **To prevent retention time problems, flush with at least 50 mL of a solvent compatible with the cleaning solution.** • **High-purity formic acid is expensive.** • **Do not leave wash solution overnight when not flowing.** • **Do not mix 100% organic with strong acid.**	• Standard, or "acid wash" • Cleans the LC inlet for use with acidic mobile phases. • Formic acid is volatile.
	• Final rinse: Prime with ultra-pure water through the lines used for cleaning for several minutes.				
2	• 80% 2-isopropanol (IPA) • 10% water • 10% ammonium hydroxide (concentrated)	• ~29% ammonium • ~56.6% ammonium hydroxide	An aggressive cleaning mixture for compounds more soluble in base - for example, phthalates and PEG-containing compounds, including detergents (Triton X-100, Triton X-114, Brij 35).	• **Never use in ACQUITY M-Class, nanoACQUITY, or any system containing silica capillary tubing that dissolves >pH10.** • **To prevent retention time problems, flush with at least 50 mL of solvent compatible with the cleaning solution.** • **Do not leave wash solution overnight when not flowing.** • **Do not mix 100% organic with strong acid.**	• "Basic wash" • Cleans the LC inlet for use with basic mobile phases. • Ammonium hydroxide is volatile.
	• Final rinse: Prime with ultra-pure water through the lines used for cleaning for several minutes.				

Table 1: Recommended cleaning mixtures for LC inlets[1]

Cleaning conditions	Solvent mixtures (v/v)	Stock concentration	Description	Cautions	Comments
3	• 30% phosphoric acid (concentrated) • 70% water	~85% Phosphoric acid	An aggressive mixture for removing organic contaminants.	• **To prevent burns from splattering solvent, slowly add the acid to water while stirring.** • **Not compatible with mass spectrometers.** • **To prevent retention time problems, flush until you reach neutral pH (pH=7) with a solvent compatible with the cleaning solution (for example, 100% water).** • **Do not leave wash solution overnight when not flowing.** • **Phosphate is insoluble in high-organic concentrations. Always flush phosphate with large quantities of water.** • **Do not mix 100% organic with strong acid.**	• "Phosphoric acid wash" • Phosphoric acid works as both a strong acid and an oxidizing agent, destroying compounds. • Not volatile and not compatible with mass spectrometers. • Loss in sensitivity. • You might see clusters at 98.
	• Final rinse: Prime with ultra-pure water through the lines used for cleaning for several minutes.				
4	100% IPA	N/A	Neutral, hydrophobic compounds	• **Do not mix 100% organic with strong acid.** • **Inorganic salts containing sodium, potassium, phosphate precipitates.**	N/A

1. Use these cleaning solutions only to clean LC inlet components that have been identified as the source of contamination (see Troubleshooting Contamination, page 11). Do not use any of these cleaning solutions to clean a column. Refer to the care and use instructions provided by the column manufacturer.

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.

Caution: If you have flushed with phosphoric acid, you must flush with water before mobile phase.

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 for the fixed loop sample manager, or the largest volume available for the FTN sample manager) of the cleaning solution. Then return to the mobile phase and wash solutions required for analysis and flush thoroughly.

Cleaning the column

See Cleaning columns, page 17.

Cleaning MS Systems

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 is to dissolve contamination from the surface of the MS component. To avoid recontaminating clean or new components, use a methodical cleaning process. Sometimes you clean or replace one component at a time, working from the first component to come into contact with solvent to the last component. In other cases, it is better to clean all suspected components simultaneously. Be sure to use ultra-pure solvents and clean glassware (see Preventing contamination, page 3).

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

Caution: To avoid contact with toxic contaminants, wear gloves and eye protection.

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

Note: Effective removal of contamination can require several sonication steps, using fresh cleaning solvent at each step. Each sonication step should last between 15 minutes to 1 hour. Longer times may be required if the flow rates are low (for example, M-Class).

3. If these solutions fail to reduce contamination levels, replace the parts.

Caution: To avoid damaging PEEK components and T-Wave assemblies, do not sonicate in chlorinated solvents, hexane, acetone, or acids.

4. Be sure to rinse the glassware thoroughly and use fresh, clean solvent between each step.
5. After final sonication, remove the MS component from the cleaning solution. Quickly dry the component with a strong stream of clean, dry nitrogen.

Caution: Fast, thorough drying is necessary to prevent solvent spots, which can affect MS performance.

Fluidics

After decontaminating the rest of the plumbing, strip and clean the fluidics 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.

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.

Important: Ensure that solvent quality and glassware cleanliness (Select, prepare, and handle solvents correctly, page 3).

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

API probes

Replace or rebuild contaminated API probes rather than cleaning or flushing it.

LC tubing

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

Reference Information

Solvent considerations

Possible effects of low-quality solvents

Not all assays require a high-grade solvent. When selecting a solvent, choose the highest-purity solvent appropriate for your application. Consider the solvent contaminant types and consequences. Table 2 shows the possible effects of low-quality solvents on your assay.

Table 2: Possible effects of low-quality solvents

Solvent contaminants	Possible problems	Analytical considerations	Preventive actions
Particle content less than (<) 0.2 microns	• Increased system back pressure and high-pressure shutdowns • Clogged tubing and columns • Damage to pumps and injector causing leaks and non-reproducible results	More down time and less productivity	• Use only high purity solvents that are filtered by the vendor to remove particulates greater than 0.2 microns. • Wear gloves to eliminate oils from skin and hand creams. • Do not refilter vendor-filtered solvents.
Micro-organisms - bacteria, algae, fungus	• Behave like particulates • Produce organic and ionic contaminant compounds	• More down time and less productivity to remove contamination • Loss of sensitivity	• Use fresh aqueous mobile phases. • Wear gloves to eliminate oils from skin and hand creams. • Use fresh, high purity, filtered water (<0.2-micron filter). • Never top-off bottles; empty, clean, and refill with fresh mobile phase. • Never store the system in high aqueous mobile phase. • Use appropriate caps on mobile phase bottles.

Table 2: Possible effects of low-quality solvents

Solvent contaminants	Possible problems	Analytical considerations	Preventive actions
UV absorbing organic compounds (detectable in TUV, PDA)	• Noisy or changing (sloping) baseline • Ghost peaks • Loss of sensitivity • Loss of linearity • Shifting retention times • Abnormal peak shapes	• For high sensitivity and detection of low concentrations of analytes, a smooth, flat baseline is desired for the highest signal-to-noise ratio. A high signal-to-noise ratio improves the reproducibility of quantitation. • High UV background can decrease the linearity of the method at high analyte concentrations.	• Do not use plastic bottles as solvent or mobile phase reservoirs. • Wear gloves to eliminate oils from skin and hand creams. • Use vials, well plates, and caps appropriate for LC organic solvents. • Never wash LC glassware (bottles, funnels, graduated cylinders, etc.) with detergents. • Have dedicated LC glassware. • Do not refilter vendor-filtered solvents. Filters or filtering apparatus can add contaminants.

Table 2: Possible effects of low-quality solvents

Solvent contaminants	Possible problems	Analytical considerations	Preventive actions
Ionizable organic compounds detectable in the mass spectrometer	• Noisy or high baseline • Loss of sensitivity • Ghost peaks • Shifting retention times • Abnormal peak shapes	• Contamination from low mass ions, PEG compounds, and phthalates (plasticizers) can cause: • Loss of sensitivity, lower signal-to-noise ratio due to ion suppression • Spectral peaks at the same masses as the analytes	• Do not use plastic bottles as solvent or mobile phase reservoirs. • Plastics are the source of phthalates and are found everywhere including in air, water, and solvents. • Wear gloves to protect skin from oils and hand creams containing PEG. • Use vials, well plates, and caps appropriate for LC organic solvents. • Never wash LC glassware (including bottles, funnels, graduated cylinders) with detergents or in a dishwasher. • Have dedicated LC glassware. • Do not refilter vendor-filtered solvents. Filters or filtering apparatus can add contaminants.

Table 2: Possible effects of low-quality solvents

Solvent contaminants	Possible problems	Analytical considerations	Preventive actions
Inorganic ions	• Adduct for formation with cations (Na+, K+) detected in the mass spectrometer • Loss of sensitivity • High pressure and clogging • Cluster formation (for example NaCl clusters, delta mass = 57.96) • Appearance of red deposits, “rust”, from iron Fe+3	• Loss of sensitivity due to adduct formation which splits the analyte spectral peak into several peaks with different masses (for example, Na+ increases m/z by 22 amu). • Inorganic compounds are not soluble in high organic solvent concentrations, resulting in high pressure and clogging, or clusters.	• Use fresh, high-purity, filtered (<0.2-micron filter) water and solvents. • Use high-purity mobile phase additives. • Use borosilicate (hardened) glass for mobile phase reservoirs (for example, Pyrex® or Schott®). • Do not use original solvent bottles for mobile phases containing acids, bases, or buffers. They cause ions to leach from the glass.
Gases	Unwanted spectra from impurities, for example oils	Poor-quality gases introduce background contamination.	• Obtain gases that meet the mass spectrometer site requirements (nitrogen 95% pure or better, 99.5% pure for IMS or GC; argon 99.997% pure; helium 99.5% pure). • Use a nitrogen generator that has particles <0.01 microns, no phthalates, no liquids (for example, water). • Use a well-maintained nitrogen generator. • Gas plumbing must be chemically cleaned copper, medical-grade stainless steel with no soldered or brazed joints to minimize metal ion contamination.

Solvent purity recommendations

A high-purity solvent meets the criteria shown in Table 3.

Note: Table 3 values were obtained from known usable LC/MS solvents. Some brands have certificates of analysis indicating higher purity than these values.

Table 3: LC/MS solvent purity recommendations

Parameter	Suggested level			
	Acetonitrile	**Methanol**	**Isopropanol**	**Water**
Assay	≥99.9%	≥99.9%	≥99.9%	≥99.9%
Filtered	≤0.2 microns	≤0.2 microns	≤0.2 microns	≤0.2 microns
Aluminum (Al)	≤25 ppb	≤50 ppb	≤20 ppb	≤50 ppb
Calcium (Ca)	≤50 ppb	≤50 ppb	≤50 ppb	≤50 ppb
Iron (Fe)	≤20 ppb	≤20 ppb	≤20 ppb	≤10 ppb
Lead (Pb)	≤20 ppb	≤20 ppb	≤20 ppb	≤10 ppb
Magnesium (Pb)	≤20 ppb	≤20 ppb	≤20 ppb	≤10 ppb
Potassium (K)	≤50 ppb	≤50 ppb	≤50 ppb	≤50 ppb
Sodium (Na)	≤50 ppb	≤50 ppb	≤50 ppb	≤50 ppb
Residue after evaporation	≤25 ppb	≤25 ppb	≤25 ppb	≤25 ppb
Water	≤0.01%	≤0.05%	≤0.05%	N/A

Recommended water purification process

Ultra-pure water is sterile and contains no particulates, no detectable ionizable compounds, and no UV-absorbing compounds. The purification process must include *all* 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 ionizable compounds)
e. A pharmaceutical-grade 0.2-µm membrane filter (to remove any remaining particulates)

Caution: House deionized (DI), reverse-osmosis (RO), and distilled water do not meet these criteria.

Caution: If using a purification system, you must perform regular maintenance on it.

Other considerations

- If outlet lines have been without flow for more than 24 hours, flush them 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.
- 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.

Gloves

Order gloves

Table 4: Nitrile gloves

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

How to put on gloves

Open the gloves by unfolding the packaging leaves until the cuffs are exposed (Figure 2).

Figure 2 - 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.

Major contaminants and their sources

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

Polyethylene glycol (PEG) or PEG-like materials

PEG (Figure 3) is a synthetic polymer produced in a range of molecular weights. Common sources of PEG contamination include:

- Organic solvents (methanol, 2-propanol, acetonitrile, water)
- Mass spectrometer calibration solution
- Hand cream and other personal care products
- Detergent (Triton X-100, glassware detergents, and so on)
- Cutting solutions in machining
- Column manufacturing

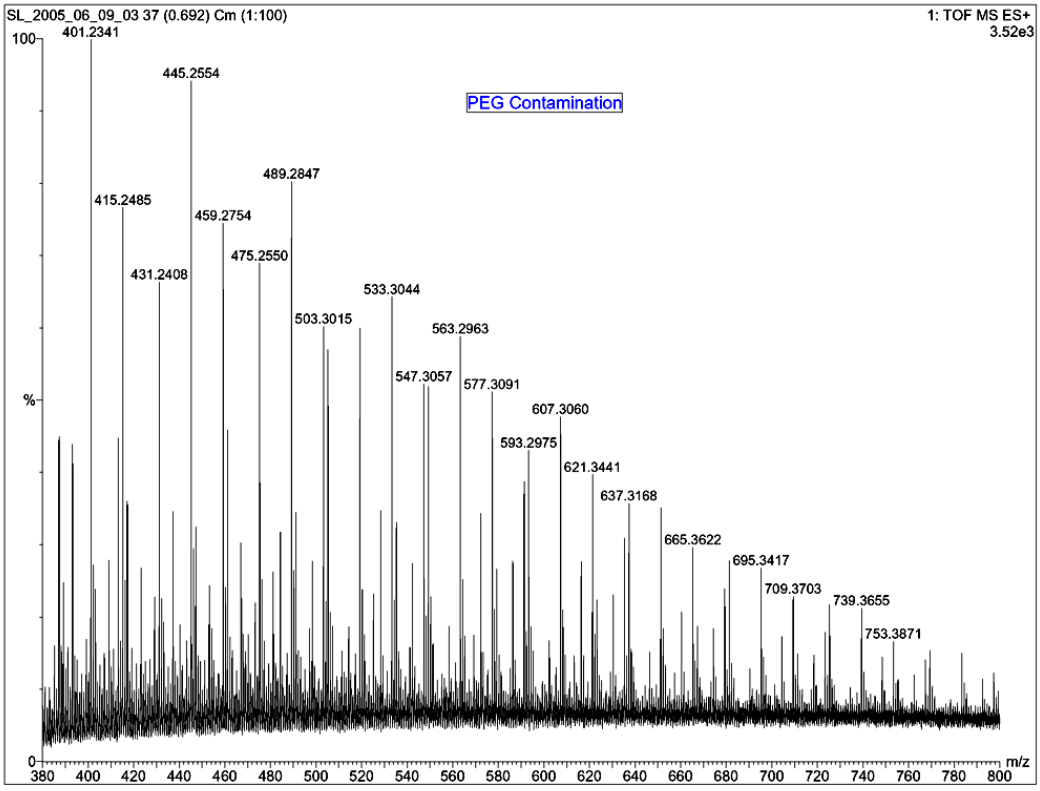


Figure 3 - PEG contamination spectra (series of mass peaks separated by 44 Da)

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 can differ, depending on the number of acetates in the cluster. The upper spectra are based on the MassLynx isotope model.

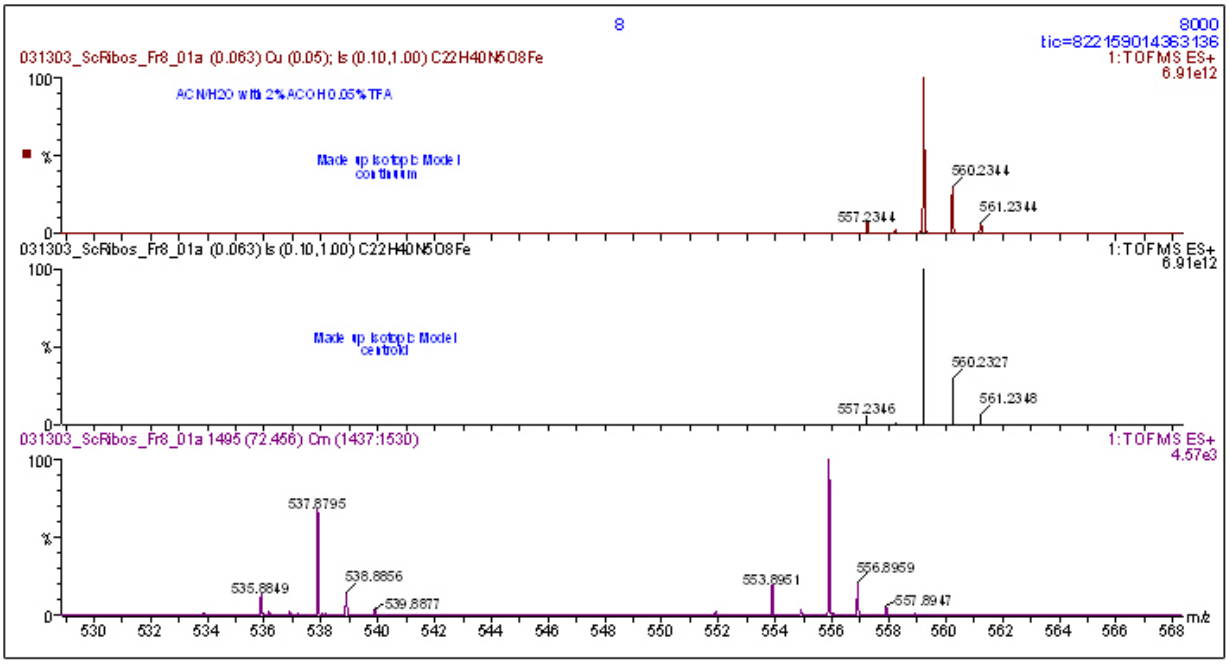


Figure 4 - Fe contamination spectra

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).

Diisooctyl phthalates can form the following adducts:

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

Slip agents (amides)

Avoid using components packed in plastic bags containing slip agents, or amides (Figure 5). The three most commonly used amides are:

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

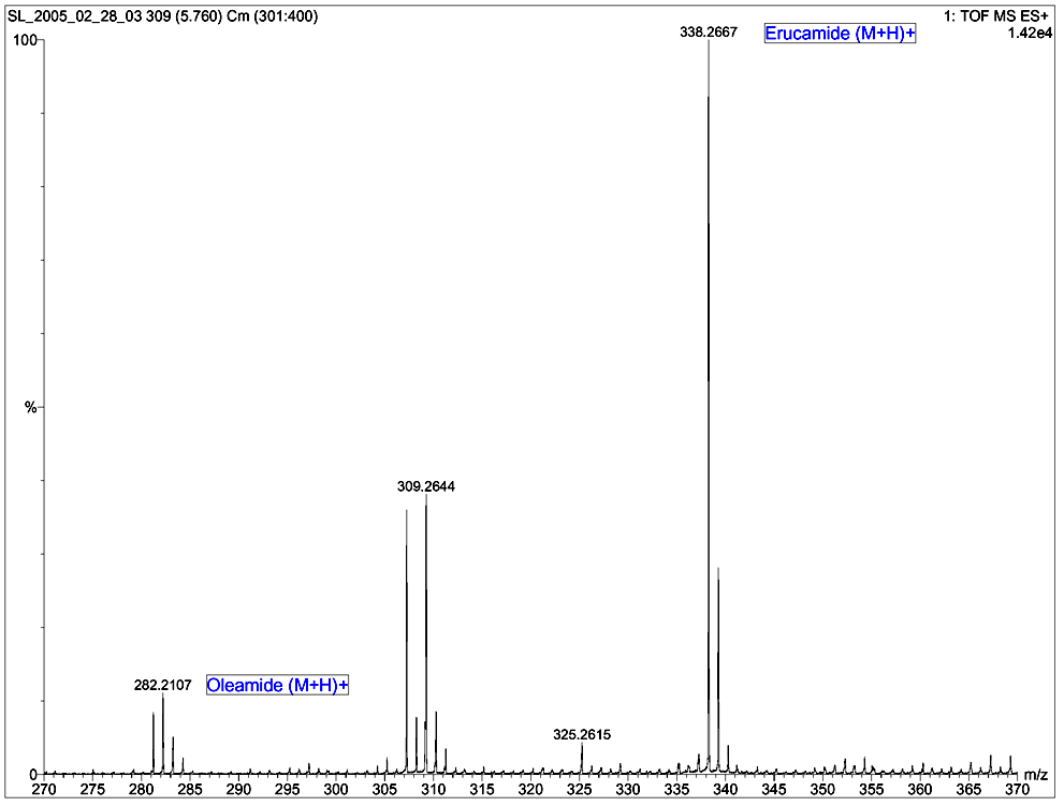


Figure 5 - Contamination spectrum of oleamide and erucamide in zip bag

Contaminant database

For a comprehensive list of major contaminants, see PEG master list and Background Ion Master List.