

# LCMS System Maintenance Tips and Tricks

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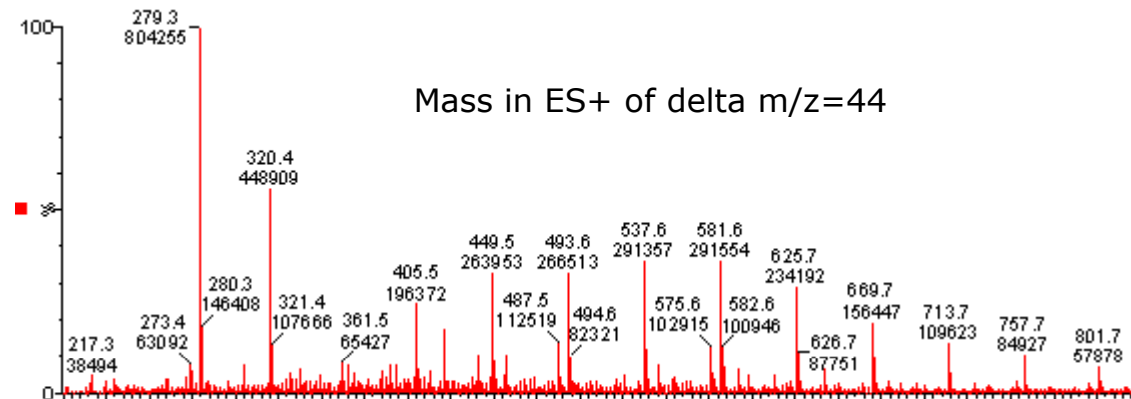


# Contamination

- Polyethylene Glycol (PEG) or PEG-Like Materials
- Metal ions
- Phthalates
- Siloxanes
- Grease /oils
- Surfactants

# Polyethylene Glycol (PEG)

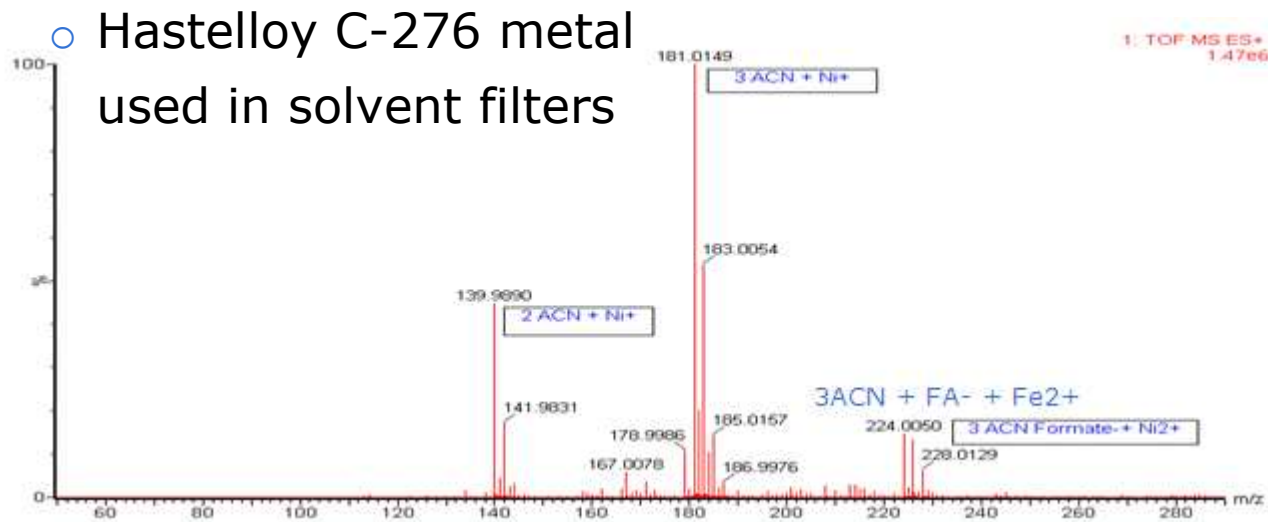
- Description
  - Polymer series



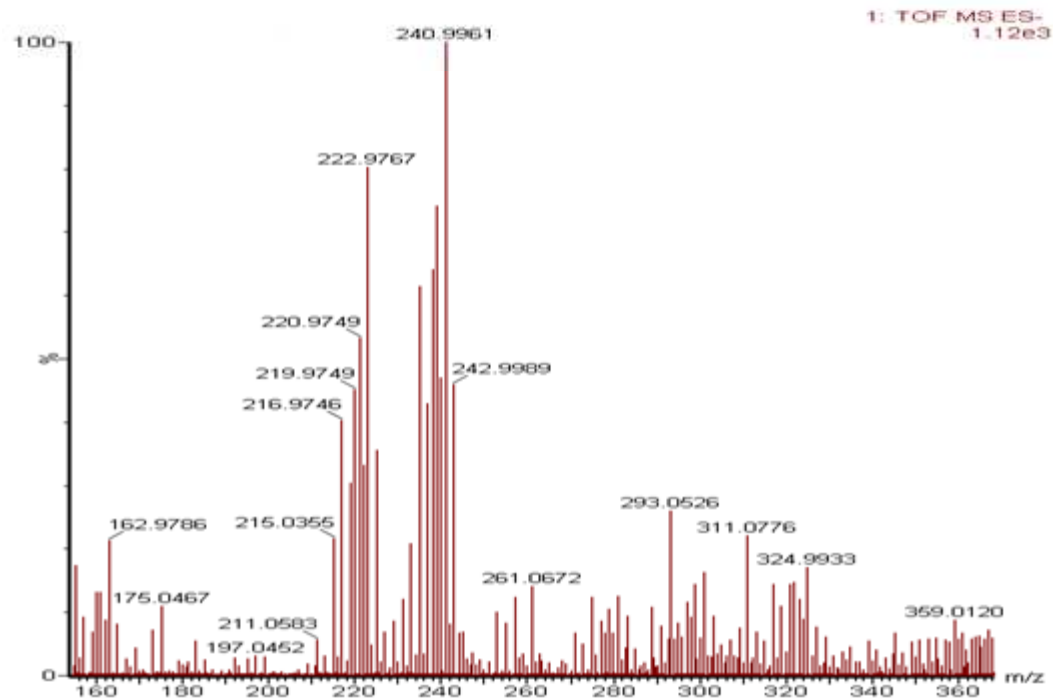
- Sources of PEG
  - Organic solvents
  - MS calibration solutions
  - Hand cream
  - Sample detergents
  - Glassware (dishwashing detergents)
  - Cleaning solutions from manufacturing

# Metal Ions

- Sodium and Potassium
- Iron
  - Sources
    - Water and acetonitrile
    - Acetic acid (lower in formic acid)
    - Non-passivated stainless steel parts
- Nickel
  - Source



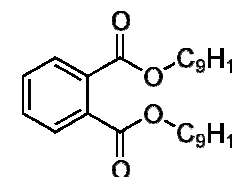
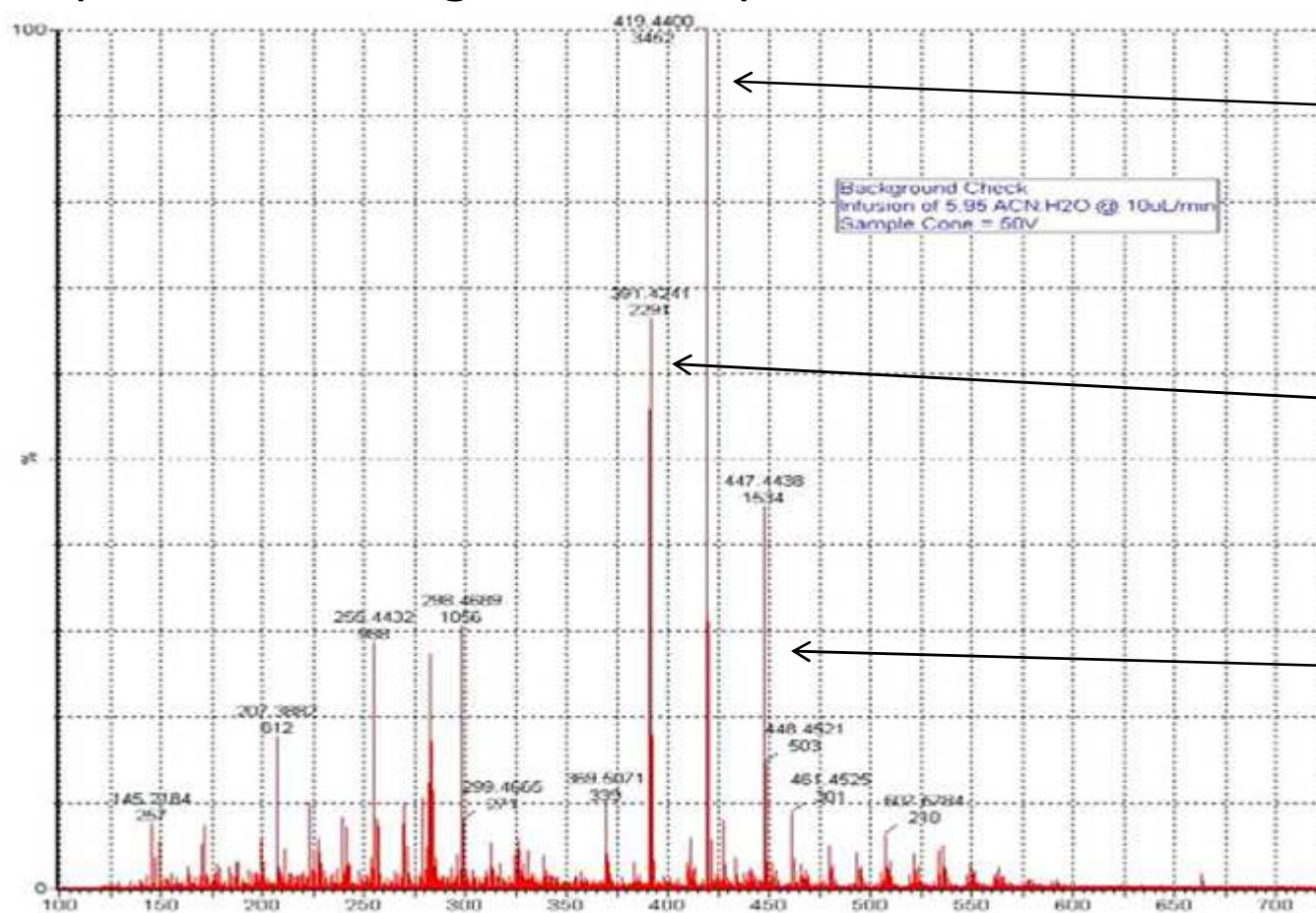
- Molybdenum
  - Molybdenum adducts of ACN + FA + H<sub>2</sub>O
  - m/z = 240
  - Sources
    - Hastelloy C-276 metal used in solvent filters



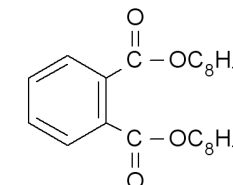
- Plasticizer
- Common phthalates include:
  - Di-2-ethylhexyl phthalate (DEHP) [M+H] = 391
  - Diisodecyl phthalate (DIDP) [M+H] = 447
  - Diisononyl phthalate (DINP) [M+H] = 419
  - Diisooctyl phthalate (DIOP) [M+H] = 391
- Adducts formed for Diisooctyl phthalate (DIOP)
  - [M+Na]<sup>+</sup> = 413
  - [M+K]<sup>+</sup> = 429
  - [2M+NH<sub>4</sub>]<sup>+</sup> = 798
  - [2M+Na]<sup>+</sup> = 803

— Phthalic anhydride m/z = 149 prevalent fragment observed
- Sources
  - Water and organic solvents that have come in contact with plastics
  - Extracted from plastic tubing, water storage containers, vials, caps
  - Laboratory air

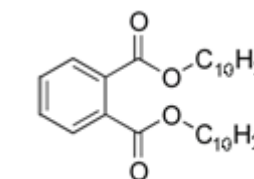
- Spectra showing common phthalate contaminants



Diisononyl phthalate (DINP)  
C<sub>26</sub>H<sub>42</sub>O<sub>4</sub>

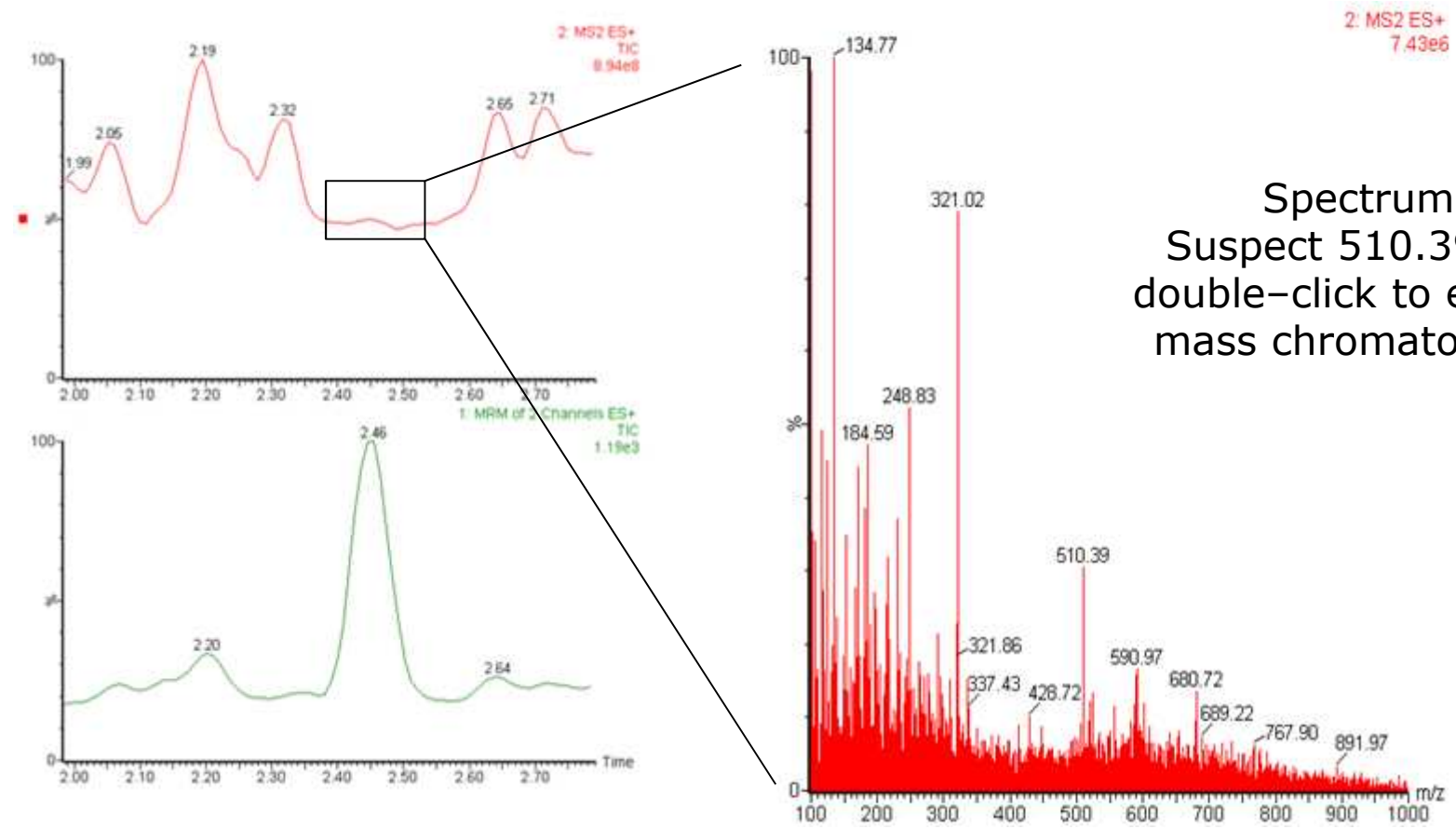


Diisooctyl phthalate (DIOP)  
C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>



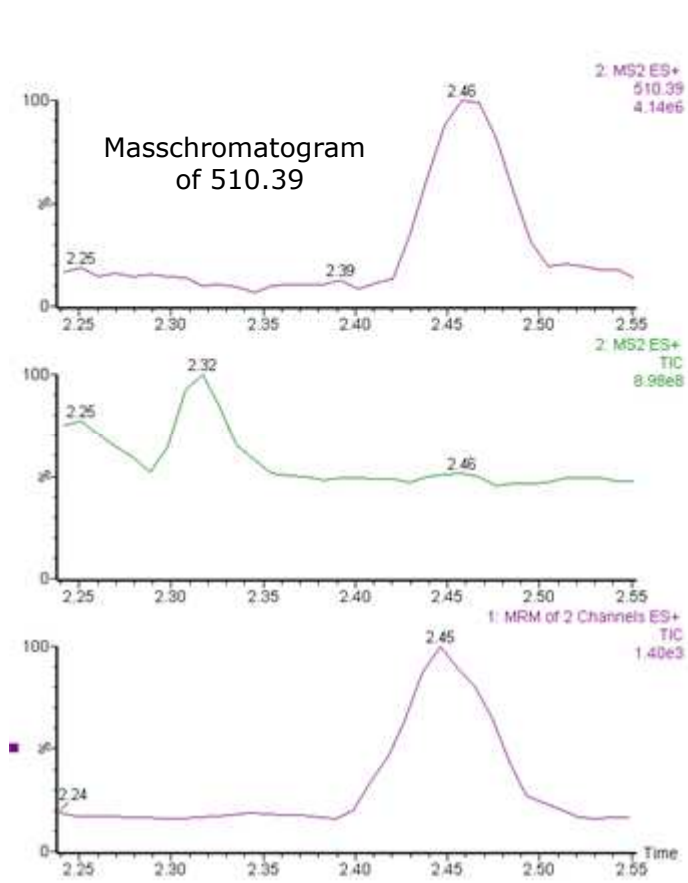
Diisodecyl phthalate (DIDP)  
C<sub>28</sub>H<sub>46</sub>O<sub>4</sub>

# MRM chromatograms combined with MS scan

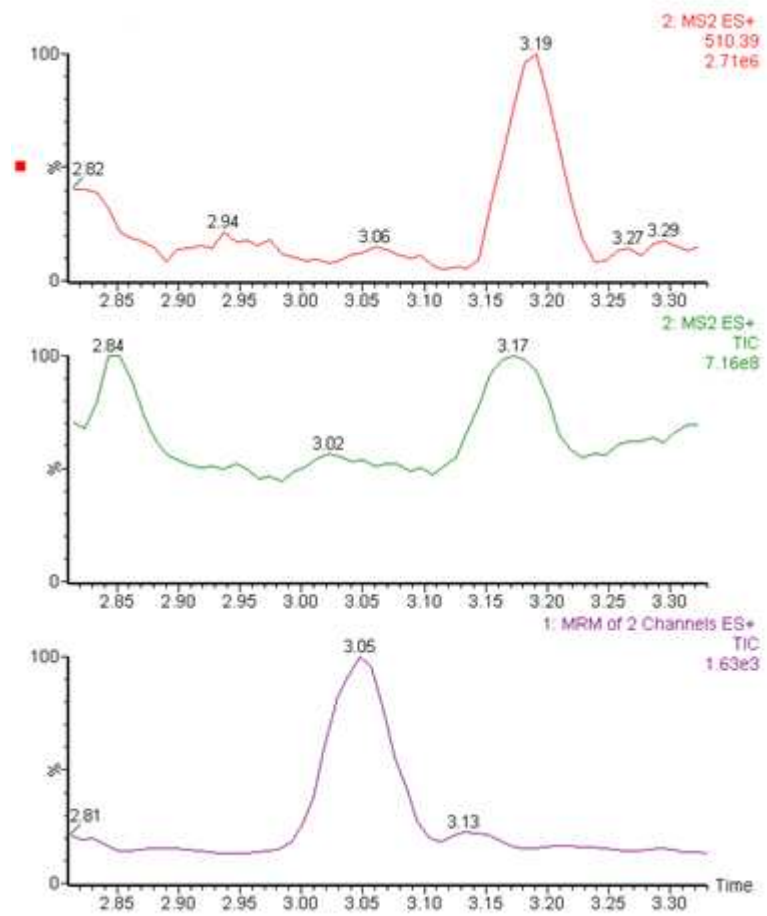


MRM chromatogram + MS chromatogram  
Extract spectra at 2.45 minutes

# Reduce ion suppression by separating contaminant from analyte



510.39 ion elutes at same r.t. as compound in MRM channel



Improve separation by lower gradient slope  
 ⇒ intensity increases 40% on MRM  
 ⇒ due to less ion suppression

## ■ Solvents and additives

- Some HPLC grade solvents might contain PEG. PEG is not seen by a UV detector, but will be observed using an MS. Always read the label
- Some HPLC grade solvents contain metal ions
- Fresh solvents are better than previously opened bottles
- Additives should also be pure. 90% formic acid solution contains 10% other compounds! Analytical grade (>99%) is recommended

## ■ Vials, caps, well plates

- Vial caps with septa may contain plastics or adhesives
- Well plate glue-on foil covers may leach adhesives



*Certified LCGC-vials,  
tested for chemical cleanliness*

## ■ Glassware

- Washing in a common dishwashing facility can contaminate glassware with detergent residues (PEG-like compounds) and surfactants
- Rinse only with mobile phase quality solvents that will be used

## ■ Plastic containers or tubing

- Storage of solvents or water in plastic is NOT recommended

# Cleaning of contamination

- Mixture 1:
  - General wash:  
25:25:25:25 ACN:H<sub>2</sub>O:MeOH:IPA + 0.2% FA  
flush with e.g. ACN:H<sub>2</sub>O 10:90 afterwards
- Mixture 2:
  - PEG wash:  
50% ACN, 49% H<sub>2</sub>O + 1% Ammonium hydroxide  
flush with e.g. ACN:H<sub>2</sub>O 10:90 afterwards
- Mixture 3:
  - 30% Phosphoric acid
    - Flush with H<sub>2</sub>O then with mobile phase
      - **Disconnect MS and column**



# Cleaning of contamination

Step	Test	Back-ground Level	Next Action
1	Can the background ions be "tuned away". E.g, are they affected by sample cone voltage, ESI capillary voltage etc	Changes  Constant	Contamination is external to MS. E.g. HPLC system  Contamination may be on internal MS component. Go to cleaning procedures.
2	Using a syringe pump fitted, directly infuse a sample of the mobile phase solutions (extracted from those solutions currently in use by the HPLC)	High  Low	a. Solvent, water, and or acid contaminated. Try different source of solvents and acid.  b. Infusion kit, bottle or MS dirty. Clean or replace.  Contamination is not in solvents or acid. Go to <b>Step 3</b> .
3	Put "clean" mobile phase mixture through each pump of the HPLC, UPLC or CapLC to the MS with no column at a low flow rate.	Higher  Lower	HPLC system is contributing contamination. Go to <b>Step 4</b> .  Not coming from the LC system. Go to <b>Step 5</b> .
4	If possible, disconnect the pump from the autosampler. Pump solvent mixture directly into MS using Inlet pump without autosampler.	High  Low	Contamination is in the pump. Go to cleaning procedures.  Contamination is in the autosampler. Go to cleaning procedures.
5	Pump mobile phase mixture through the parts of the system, adding them one at a time – tubing, filter(s), column(s).	High	The lasted added component contains the contamination Go to cleaning procedures.

# Cleaning of contamination

Table 2: Recommended Cleaning Mixtures for LC

	LC Mixture 1 <sup>a</sup>	LC Mixture 2 <sup>a</sup>	LC Mixture 3 <sup>a</sup>	LC Mixture 4 <sup>a</sup>
<b>Purpose</b>	General purpose solution for nano-ACQUITY 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
<b>Caution</b>			<ul style="list-style-type: none"> <li>• <b>HIGH-PH WASH DISSOLVES SILICA ABOVE PH=10. IN SYSTEMS WITH FUSED SILICA AND GLASS COMPONENTS, SUCH AS NANOACQUITY, DO NOT USE MIXTURE 3.</b></li> <li>• <b>AFTER CLEANING WITH ACID OR BASE, FLUSH WITH ULTRAPURE WATER UNTIL THE PH IS NEUTRAL (ABOUT PH=7) BEFORE CONNECTING TO A DETECTOR.</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>USE AS LAST RESORT</b></li> <li>• <b>DO NOT USE MIXTURE 4 WITH ORGANIC SOLVENTS.</b></li> <li>• <b>DO NOT USE MIXTURE 4 WITH NANOACQUITY.</b></li> <li>• <b>DO NOT USE MIXTURE 4 TO CLEAN SEAL-WASH LINES.</b></li> <li>• <b>REMOVE HASTELLOY SINKERS BEFORE CLEANING WITH PHOSPHORIC ACID</b></li> <li>• <b>AFTER CLEANING WITH ACID OR BASE, FLUSH WITH ULTRAPURE WATER UNTIL THE PH IS NEUTRAL (ABOUT PH=7) BEFORE CONNECTING TO A DETECTOR.</b></li> </ul>
<b>Mixture</b>	<ul style="list-style-type: none"> <li>• 100% 2-propanol (isopropyl alcohol, or IPA)</li> </ul>	<ul style="list-style-type: none"> <li>• 25% acetonitrile</li> <li>• 25% methanol</li> <li>• 25% 2-propanol</li> <li>• 25% water</li> <li>• 0.2% formic acid</li> </ul>	<ul style="list-style-type: none"> <li>• 50% acetonitrile</li> <li>• 49% water</li> <li>• 1% ammonium hydroxide</li> </ul>	<ul style="list-style-type: none"> <li>• 30% phosphoric acid</li> <li>• 70% water</li> </ul>

<sup>a</sup> CAUTION: DO NOT INTRODUCE ANY CLEANING SOLUTIONS INTO THE MS SYSTEM OR COLUMN.

Table 3: Recommended Cleaning Mixtures for MS

	MS Mix 1 <sup>a</sup>	MS Mix 2 <sup>a</sup>	MS Mix 3 <sup>a</sup>	MS Mix 4 <sup>a</sup>	MS Mix 5 <sup>a</sup>
<b>Purpose</b>	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.
<b>Caution</b>				<ul style="list-style-type: none"> <li>• <b>DO NOT SONICATE PEEK COMPONENTS OR T-WAVE ASSEMBLIES IN MIXTURE 4.</b></li> <li>• <b>AFTER SONICATING WITH MIXTURE 4, BE SURE TO SONICATE IN MIXTURE 3.</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>DO NOT SONICATE PEEK COMPONENTS OR T-WAVE ASSEMBLIES IN MIXTURE 5.</b></li> <li>• <b>BE SURE TO FOLLOW THE CORRECT SOLVENT SEQUENCE WHEN WASHING WITH MIXTURE 5 OR OTHER SOLVENTS THAT ARE IMMISCIBLE WITH WATER.</b></li> </ul>
<b>Mixture</b>	<ul style="list-style-type: none"> <li>• Water</li> </ul>	<ul style="list-style-type: none"> <li>• Organic solvent such as methanol, acetonitrile, or 2-propanol</li> </ul>	<ul style="list-style-type: none"> <li>• 50% organic solvent</li> <li>• 50% water</li> </ul>	<ul style="list-style-type: none"> <li>• 45% acetonitrile or methanol</li> <li>• 45% water</li> <li>• 10% formic acid</li> </ul>	<ul style="list-style-type: none"> <li>• Chlorinated solvents, hexane, or acetone</li> </ul>

<sup>a</sup> REFER TO YOUR DETECTOR'S OPERATOR'S MANUAL FOR SPECIFIC ADVICE ON CLEANING MS COMPONENTS.

# Reference information

Waters  
THE SCIENCE OF WHAT'S POSSIBLE.™

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

715001307, Rev. E



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### Controlling Contamination in UPLC/MS and HPLC/MS Systems

Support Number: SSPR10032452  
Document Revision: E  
Content Type: Self Service Procedures  
Part Number: 715001307  
Related Products: ACQUITY UPLC  
nanoACQUITY UPLC  
717plus Autosampler

*Controlling Contamination in UltraPerformance LC/MS and HPLC/MS Systems* lists the procedures necessary to control contamination in LC/MS systems. It includes sections on:

- Preventing contamination
- Troubleshooting contamination
- Cleaning to eliminate contamination
- Major contaminants and their sources

Two tables of related information are also attached:

*Background Ion List* provides the mass-to-charge ( $m/z$ ) ratios of common background ions, clusters, adducts, and ion series.

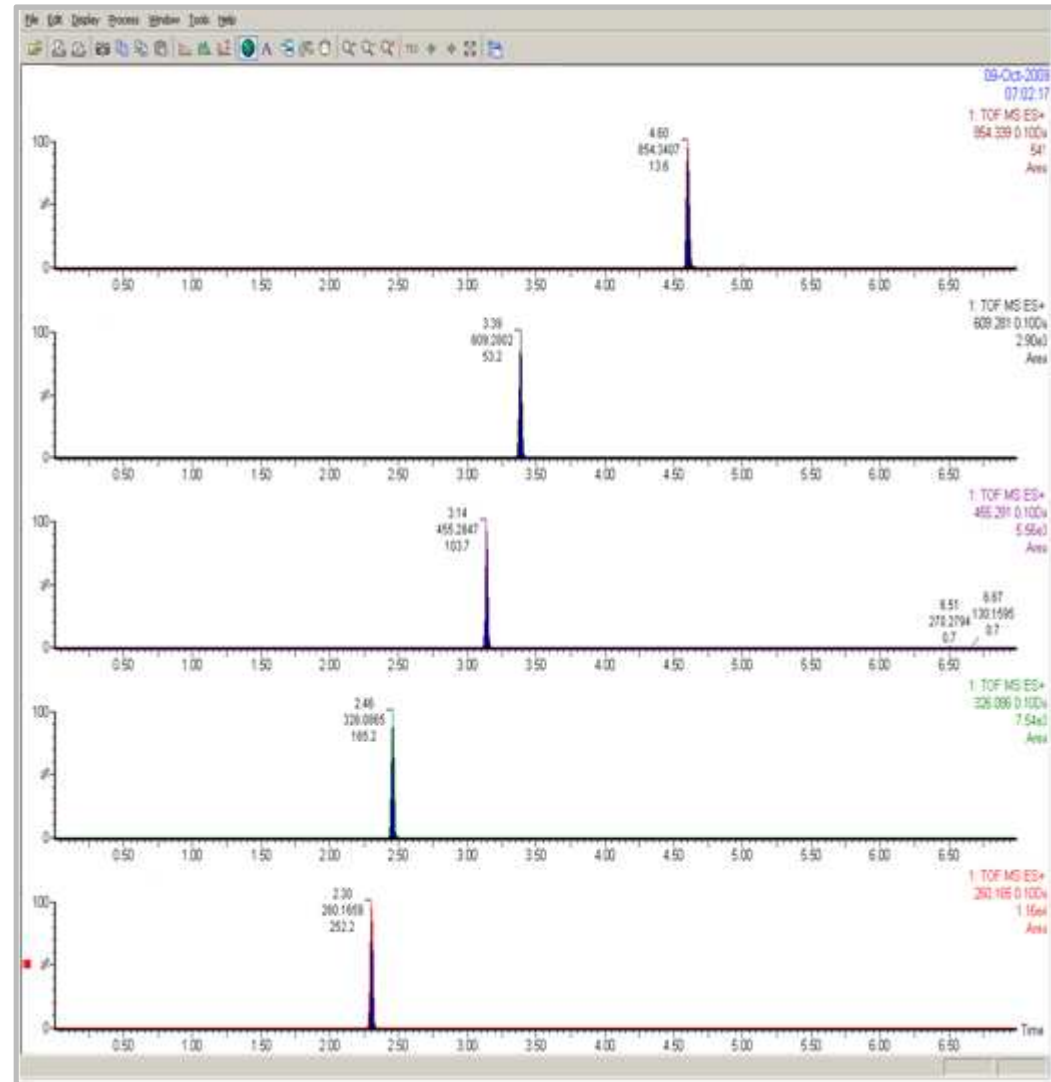
*PEG Master List* shows the monoisotopic masses of commonly occurring peaks in the positive ion spectra of polyethylene glycol (PEG) and polyethylene glycol monomethyl ether (PEGMME).

Downloadable File(s)

Title	Format	File Size
<a href="#">Controlling Contamination in UPLC/MS and HPLC/MS Systems</a>	PDF	622.13kB
<a href="#">Background Ion List</a>	PDF	89.9kB
<a href="#">PEG Master List</a>	PDF	81.7kB

# System test

- Testmix
  - Retentiontime
  - Reproducibility
  - Accurate mass



- Cleaning of sample cone
  - What samples have been introduced? Proteins? Salts?
  - Chemicals used are dependent of what has been introduced
    - H<sub>2</sub>O, MeOH, FA, IPA, ACN, DCM etc.
  - Easy contamination
    - Sonicate in MeOH for 15-30 mins, dry with N<sub>2</sub>-gas
  - General clean
    1. Sonicate in 10% FA in 50/50 MeOH/H<sub>2</sub>O for ~15 mins
    2. Rinse with H<sub>2</sub>O to remove residues of FA
    3. Sonicate in MeOH for ~15 mins
    4. Dry with N<sub>2</sub>-gas
  - Heavy contamination
    - Fiber brush pen
    - Cotton sticks

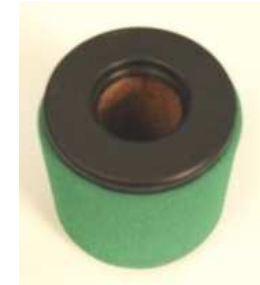


**Do NOT use fiber brush on sample cone**

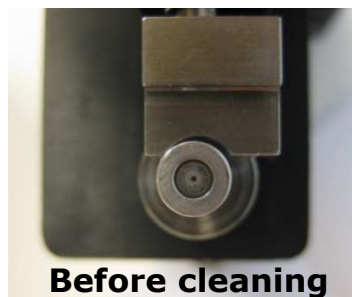
- Hexapole cleaning
  - **Easy contamination**, sonicate in MeOH for 30 mins, dry with N<sub>2</sub>-gas
  - **Heavy contamination**, sonicate in MeOH + 10% FA, rinse with H<sub>2</sub>O and sonicate in MeOH for 10 mins, dry with N<sub>2</sub>-gas
- T-Wave
  - Sonicate in MeOH for 30 mins, dry with N<sub>2</sub>-gas

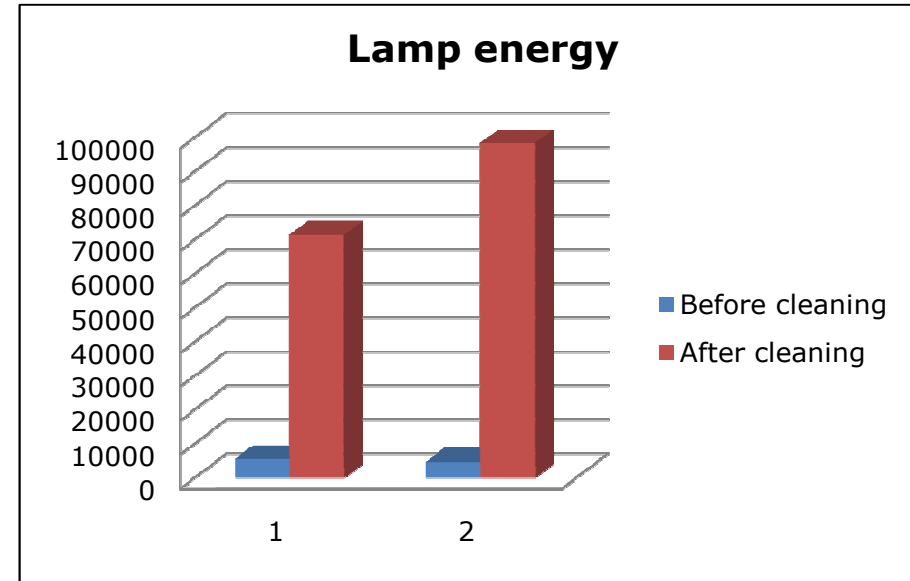
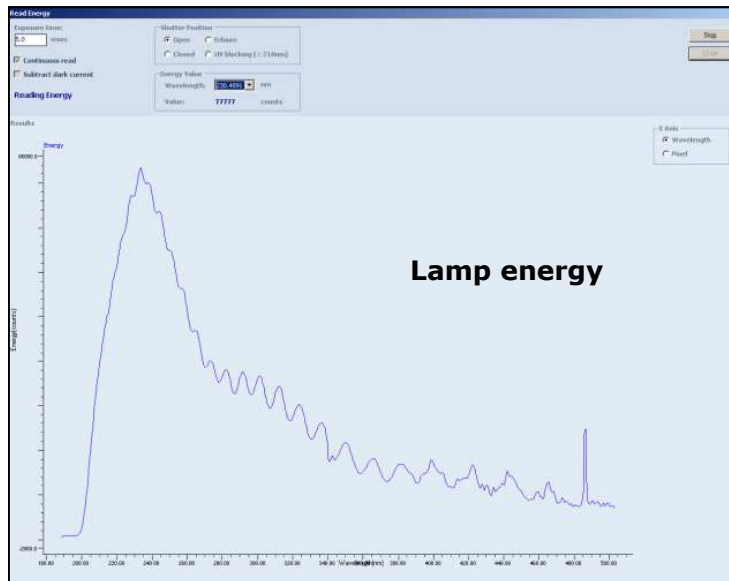


- Oilchange
  - Once a year
- Oilmist filter change
  - Once a year
- If high pH is used
  - recommended to use fully synthetic oil or otherwise the oil will need to be changed more frequent
- Cleaning of restrictor
  - When oil does not go back into pump when ballasting
  - Close Ballast and remove restrictor located in hose.  
Use a clamp on hose to avoid oil leaking from filter housing
  - Use a thin steelwire to penetrate residues.  
Sonicate for 15 mins in IPA



- Control contamination
  - Less ion suppression => increased S:N => lower LOQ
  - Less isobaric interference => Good accurate mass data
- PDA and pump
  - Flushing
    - 30% Phosphoric acid in H<sub>2</sub>O
  - Clean flowcell
    - Flush with H<sub>2</sub>O for 30 min followed by MeOH for 30 min after each batch or for 2h every weekend





- Monitor lamp-energy frequently
  - Flush or change lamp if energy is low

- Example of energy levels after flushing with phosphoric acid

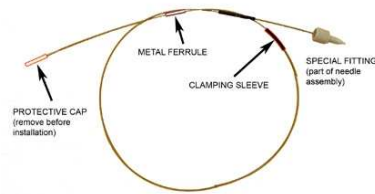
- Wash solvents (When using Partial loop with needle overfill)
  - Strong wash: 25:25:25:25 ACN:MeOH:H<sub>2</sub>O:IPA + 1-2%FA
  - Carryover issues:
    - Weak wash as above, strong wash 2% ammonia instead of FA  
=>Lower carryover => lower LOQ
- Different needles depending on samples

FEP/Metal



205000369

Peek



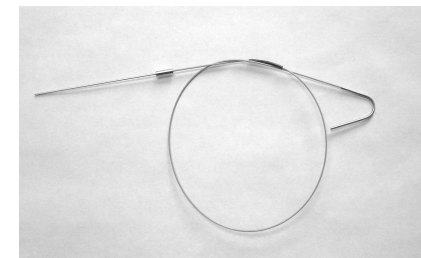
700002644

Stainless steel



205000362

PEEKsil™



700002708

- Highgrade Acetonitrile or Methanol
  - Highest chemical purity and particle-free (prefiltered by the manufacturer with 0.2 micron filter)
  - Recommended sources: *J.T. Baker®: LC/MS Grade, B&J Brand® LC/MS Grade, or Fisher: Optima® LC/MS Grade*
  - Increased uptime, seals and plungers last longer
  - Less contamination
  - **Do NOT filter already filtered solvents**
  - Always use fresh bottles, avoid putting the solvent filters on the table



- Avoid hastelloy filters (contamination-clusters with Mo, Ni)

- Stainless steel filters for Quads  
(Partnr: 700003616)

- Titanium filters for High resolution instruments  
(Partnr: 700003530)



- Use the lowest possible concentration of mobile phase additive (e.g., 0.1% formic acid, not 1%)
- Use the highest quality of additives available.
- 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

Questions?

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