# Troubleshooting Common LC/MS Contamination

Chemical contamination is one of the more common problems with LC/MS.
Try this troubleshooting path.

1. HPLC pre or trap column
2. HPLC column
3. HPLC hardware
4. Chemicals
5. ESI or APCI probe
6. Spray shield area
7. Heated capillary – reaming only removes blockages
8. Tube lens/skimmer
9. Octopoles/Multipoles
10. Trap
11. Dynode

Mostly if 8 – 11 are dirty you will see random peaks not specific masses.

**Phthalate Contamination (391, 413 798, 803)**
391 protonated dioctyl phthalate (M+H)+.
413 sodium adduct of dioctyl phthalate (M+Na)+.
798 ammoniated dimer of dioctyl phthalate [2M+NH4]+.
803 sodiated dimer of dioctyl phthalate (2M+Na)+.
This is usually from contaminated solvents. It can concentrate on the column and elute during a gradient. Follow the normal solvent checking procedures.
Glassware run through a "dishwasher" often picks up phthalate contamination. Remove this with a rinse of 30% nitric acid followed by a rinse with 2M NH4OH.
The APCI probe can retain this. Baking the APCI will eliminate this problem. Try 550 deg C for 15 min.

## +44 Series

Possible polymer contamination. If you have shot detergent containing samples on the system that could explain the background. Also PEGs and other ethoxylated polymers give +44 ion series. The PEG's could also be from the water, or extracted polymer from plastic ware/silicon coatings.

## +59 Series

And what about a +59 ion series? I'm using acetic acid in a 5% concentration. 59 Da is the mass of the acetate ion. But how acetate interact with the peptide? Is it possible to have acetate polymerization? There is another explanation for the +59 ion series?
Polymers of +59 might be iron in some form, presumably leaching from the steel in acid. We saw a lot of that before we eliminated as much metal as possible. We also saw +59 adducts onto larger, acidic peptides.

## +77 Series

I had been experiencing contamination on the Deca (ca 77 u clusters, mostly across the mass range). This appears to have resolved itself upon replacing the heated capillary and seal (kelrez ?, the soft black one). The seal was visibly "chewed-up". I don't know how this might related to the problem, but at least it appears to be gone.
Contamination peaks related to a bad heated capillary o-ring is a new one on me. I could postulate that the worn o-ring was allowing leakage into the tube lens skimmer area and the leak was also leaching something out of the o-ring. Normally the o-ring is a total block and any polymers in it would not enter the MS.

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## 615.7 and 1229.8 CHAPS

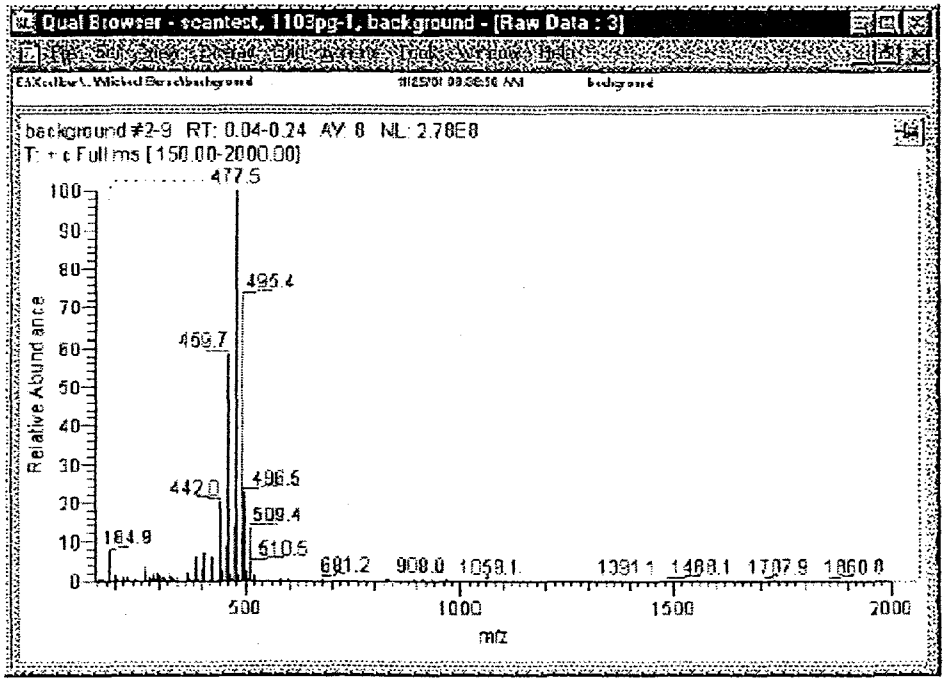
615.7 is MH+ of CHAPS and 1229.8 is (2M+H)+ in the sample. Removal can be tough. Acetone precipitation removes the excess CHAPS but there may still be an appreciable amount remaining (determined by the above ions in the mass spectrum).

CHAPS won't kill your SCX chromatography, but it will chromatograph nicely on reversed phase and you'll get an intense ion at 615 m/z (MH+).

## Nanospray Peaks (371, 445 and others)

Peaks at 371 and 445 amu are commonly observed with the nanospray probe. This may result from the silica.

A long troubleshoot for a source of contamination in Nanospray ended when the user replaced:
97044-60290 HV Emitter Nanospray ay $141 (the nanospray micro-Tee)
The spectrum was: 442.0, 459.7, 477.5, and 495.4 (see picture)
The emitter/Tee assembly had obviously become contaminated. This piece could be hard to clean as the electrode side of the Tee cannot be taken apart and assembled again correctly.




## +136 Series

Example: You have a background (contamination) with a repeating sequence of 136 amu. This could be from the chromatograph or internal to the LCQ. Identify the source by infusing methanol with a clean syringe and a new piece of tubing.

If the source of the contamination is the LCQ then try the following:

- Using the syringe pump spray acetonitrile at a relatively high flow rate and cycle the heated capillary from 150 to 270 C two times. Allow a few minutes before changing the temperature.
- Disassemble and clean the ESI head.
- Disassemble and clean the API stack.

- Remove and clean the octopoles.
- Disassemble and clean the Trap.

If the source of the contamination is the HPLC.
- Test the mobile phase for contamination by infusing at 500 uL/min with 500 uL syringe.
- To minimize contamination use fresh chemicals, particularly acids.

## Pentafluoropropionic Acid

May stick on PEEK tubing and fittings. Usually I associate contamination with areas that are poorly swept by the flowing liquid (e.g. unions).
If you have concerns about the pentafluoropropionic acid then use fused silica and steel unions. The ferrules should be kel-F. PEEK tube nuts should be ok. They do not touch the liquids.
What is the purity of the pentafluoropropionic acid? I always worry about purity of mobile phase additives.

## Water

If you see possible contamination use high grade bottled water (Burdick & Jackson HPLC grade.) Avoid ANY nanoPure or MilliQ water. The equipment may not be maintained correctly.

## 798 and 803 Contamination

This looks like phthalate contamination to me. m/z 798 is probably the gas phase dimer of dioctylphthalate
(MW 390) plus ammonium [2M+NH4]+. The m/z 803 would then be [2M+Na]+.
Sometimes this stuff can be really hard to track down. If it's coming from your HPLC it can concentrate on your column until you ramp the gradient to knock it off. I would check the solvents by infusion, that way you'll know if it's coming from your HPLC or if it's in one of your reagents

Now, if one of our buffer bottles inadvertently gets "dishwashered", we rinse well with 30% Nitric Acid followed, after a water rinse, by 2M NH4OH. This has removed MANY odd artifacts from our LC/MS/MS runs on both our LCQ's and our new DECA

## Peak Clusters at +21, -17, -35, -52

+21 is sodium, -17, -35, -52 are various losses of ammonia and water (if you look at the -17 ion you will probably see a small amount of the -18, as well).