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Dedicated to Mass Spectrometry

MS VISION TECH TIPPS SERIES
PART I — COMMON BACKGROUND IONS IN LC-MS

In this article we would like to discuss a bit about common background ions in LC-MS and how to avoid or eliminate them.

Background ions are an issue in LC-MS analysis for several reasons:

- If they appear all the time and interfere with masses of your analytes, they can complicate peak detection
- If they accumulate on the column they can cover all other ions once they come off the column at higher solvent strength
- Strong signals of background ions will require to repeat experiments after cleaning when results are supposed to be published
- In trapping systems (ion traps, orbitraps, FT-ICR) the accumulation of background ions reduces the available number of ions/charges for your analytes and thus sensitivity. With a quadrupole upfront you can reduce this, but it's better not to have background ions at all.

So where to they originate from? Background ions have many different sources. The most common ones are sample prep, solvents and ubiquitous chemicals. According to these three sources, the commonly observed background ions can be categorized by where they originate from:

The ideal strategy to eliminate background ions is

- To identify what they are. For this a number of internet based resources can be used. There are lists available sorted by mass which give you an indication what it could be. Always also consider a mixture such as e.g. sodium, potassium and PEG, which gives rise to double series with a 16 Da mass difference potentially misinterpreted as an oxidation. Databases for background ions can be found at: https://www.maconda.bham.ac.uk/browse.php and https://www.lc-ms.nl/contaminants.htm
- Try to localize where actually the contamination comes from. Is it based on the sample, the sol-

	Sample prep	Solvents	Ubiquitous chemicals
PEG based polymers (Tween, Triton, PEG)	X		
Nylon breakdown products		X	
Salt clusters	X		
Bis-(Ethylhexyl)-phthalate			X
Siloxanes			X
Plasticizers			X
Sodium adducts		X	
Potassium adducts			X (any smokers place closeby?)
Oxidation			X (older Xerox machines or laser printers!)

vents or something which hides in the system. Follow an analytical approach. E.g. if you disconnect the LC and perform an infusion measurement and the background is still there, it cannot come from the LC.

- Once you identified the localization of the contamination try to eliminate it. Try to exchange capillaries, fittings, ferrules, etc. Contaminations can stick on surfaces or in dead volumes. You need to find where it sits. If it is sample related, try a more rigorous sample cleanup. E.g. if you observe polymers in proteomics samples, run a short SDS-gel of the undigested sample and perform an in-gel digest.
- Sodium and potassium adducts can originate from poor glassware, try to exchange bottles. Ideal are PTFE bottles as they are alkali and plasticizer free. Unfortunately they are also quite expensive. If you see other metals such as iron adducts – this is a sign for corrosion somewhere and should normally not occur!
- Detergents might originate form glassware cleaned in dishwashers. Avoid those if possible!
- Nylon breakdown products can cause heavy contamination. I have observed these once as bottled water was filtered through nylon membranes. Don't do this! Buy high quality bottled water or produce 18MΩ water using a well maintained(!) water purification system.
- Siloxanes and plasticizers are particularly hard to eliminate. Siloxanes can originate from sealings and sealing material in electronics components and sources but also from floor coatings. Instruments with a relative open source design such as from ThermoFisher are particular prone to this.

  Plasticizers can originate from everywhere, plasticware, tubings, microreaction tubes, Falcon tubes, etc. During my past we had the saying "If you don't see 391 Da [Bis-(ethylhexyl)-phthalate] you are just not sensitive enough." In particular for plasticware the best approach is to buy different brands, fill them with a strong solvent such as fresh THF or Acetonitrile/DMSO, wait for two days and test them in infusion experiments. If there is no plasticizer present – stick with that brand! Pay particular interest if there is a general purchasing of these items e.g. in your university. Central procurement tries to save 2 cents per tube or tip and wrecks a 2.000€ worth sample in it…
- If you already contaminated your LC system, disconnect the column (there will be a separate article on how to clean columns) try repetitive injections of strong solvents such as DMSO to clean it and then run a saw tooth gradient for some time.
- If all that doesn't help, try to exchange capillaries, fittings, ferrules, etc. Think about deadvolumes where it could hide. Make sure you used the proper ferrule/fitting for the union! There are various different designs available and they do not necessarily fit together. This is one of the most common source of dead volumes in which contaminations can hide!
- If you are still not successful, consider an electronic issue of the system. E.g. we observed recently that several Thermo LTQ Orbitrap system showed constant but different background signals. These were interfering with the analysis. It turned out that these signals showed no isotopes and were present in MS and MS/MS. It took a while to get to the root cause, in the end it was a corrosion/ grounding issue in the electronics. Once fixed, the background was gone!

Good luck with getting rid of your background!