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LC-MS Tips & Tricks

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NEW TO LC-MS? 10 PRACTICAL TIPS THAT WILL MAKE A DIFFERENCE

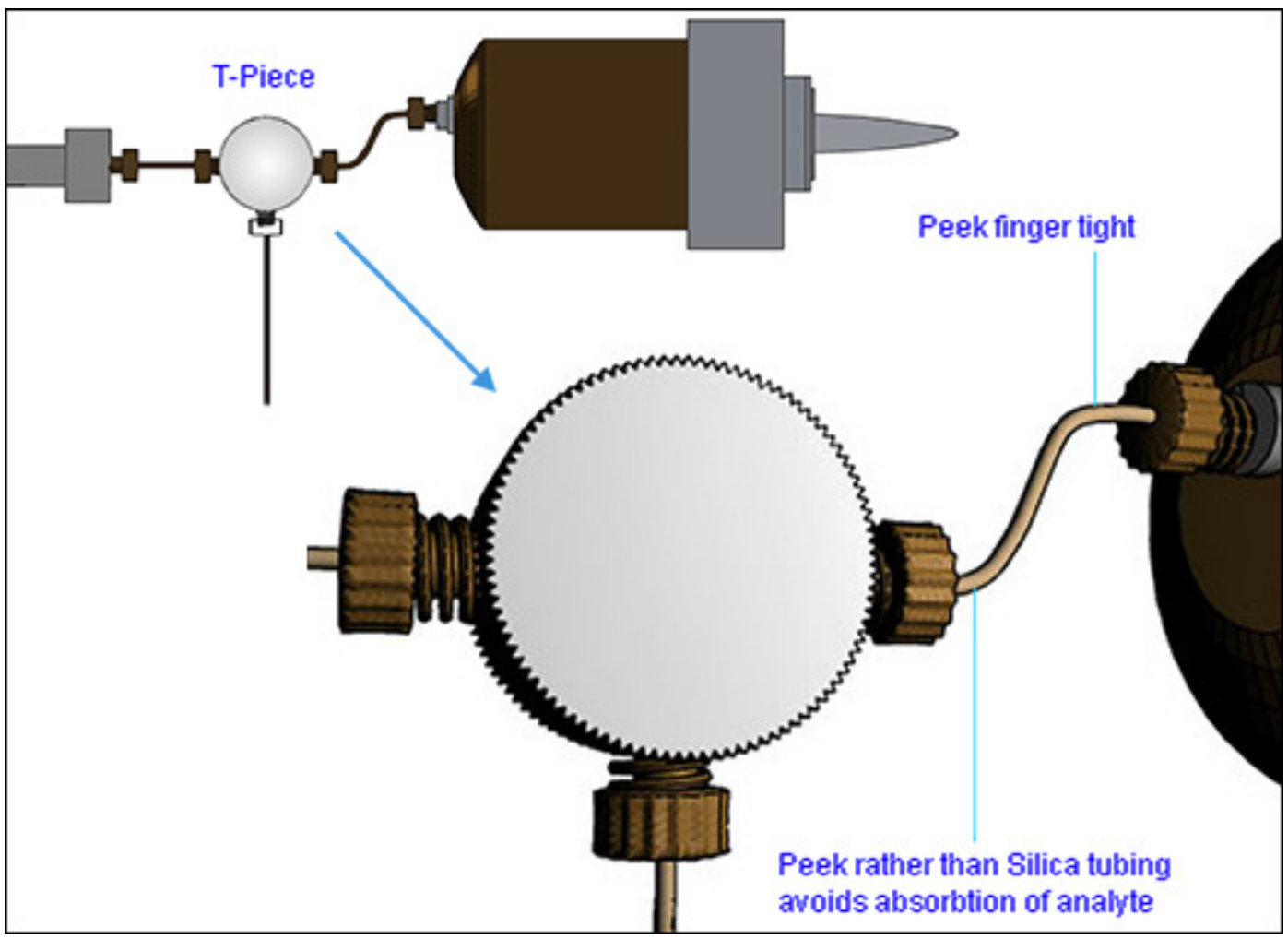
1. Contamination, contamination, contamination! LC-MS is hugely affected by contamination. Sensitivity drops rapidly and can only be restored by a vent and clean. Make sure your samples are clean – particularly for dissolved contamination. This often means using SPE.

2. Use a divert valve between the HPLC and the MS. Only introduce the mobile phase stream to the ms for the retention areas of interest. Divert everything else (particularly t0 and high organic portion of the gradient) to waste. Everything you inject into the HPLC will enter the mass spectrometer to build contamination.

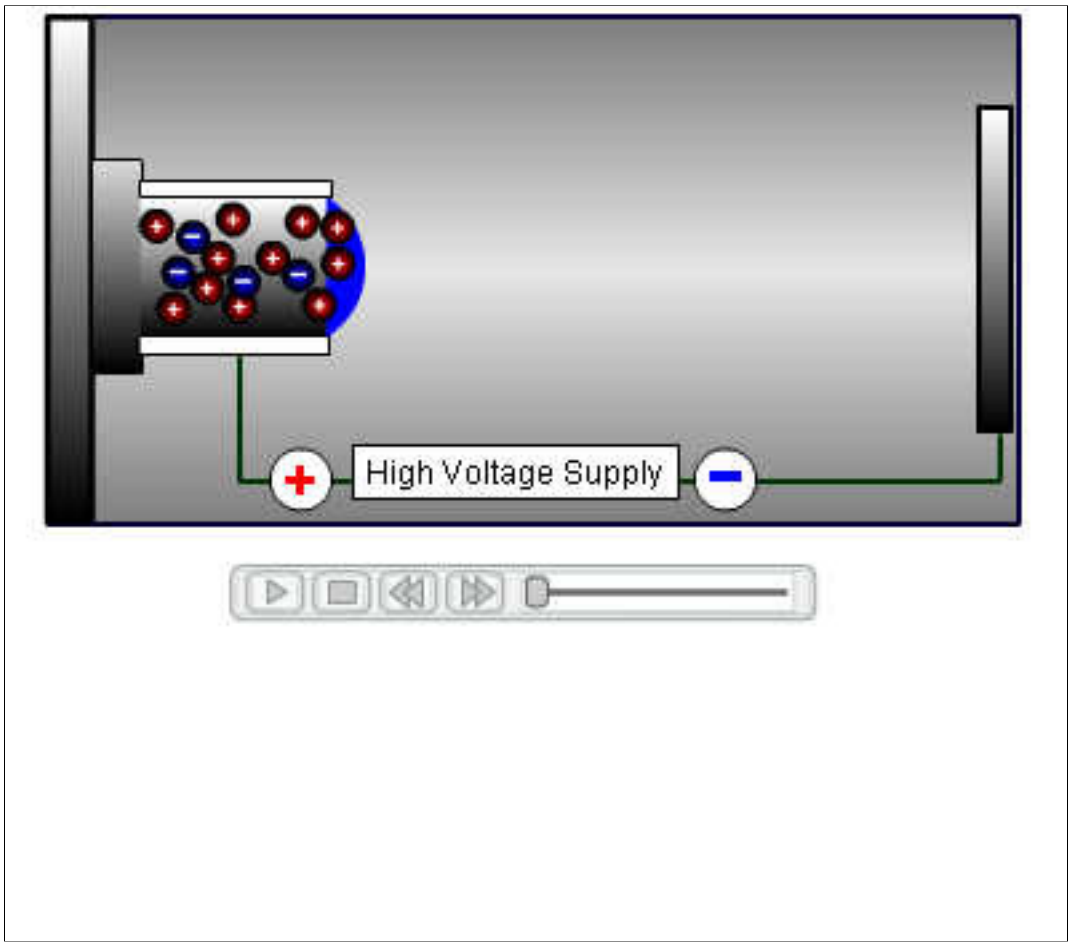
3. Vent the instrument as seldom as you can. Mass spectrometers are at their most reliable when left running. The more you vent the instrument, the more likely you are to cause other problems. The need to vent is caused by the need to clean, which is caused by contamination, which brings us back nicely to points 1 and 2.

4. Clean it all – not just one bit.

If you've gone to all the trouble of venting the instrument, take an extra few minutes to remove and clean everything up to the mass analyser. There's nothing more frustrating than venting, cleaning just the front items, then re-establishing vacuum to find sensitivity hasn't improved and having to repeat the whole process to now clean the pre-filter.

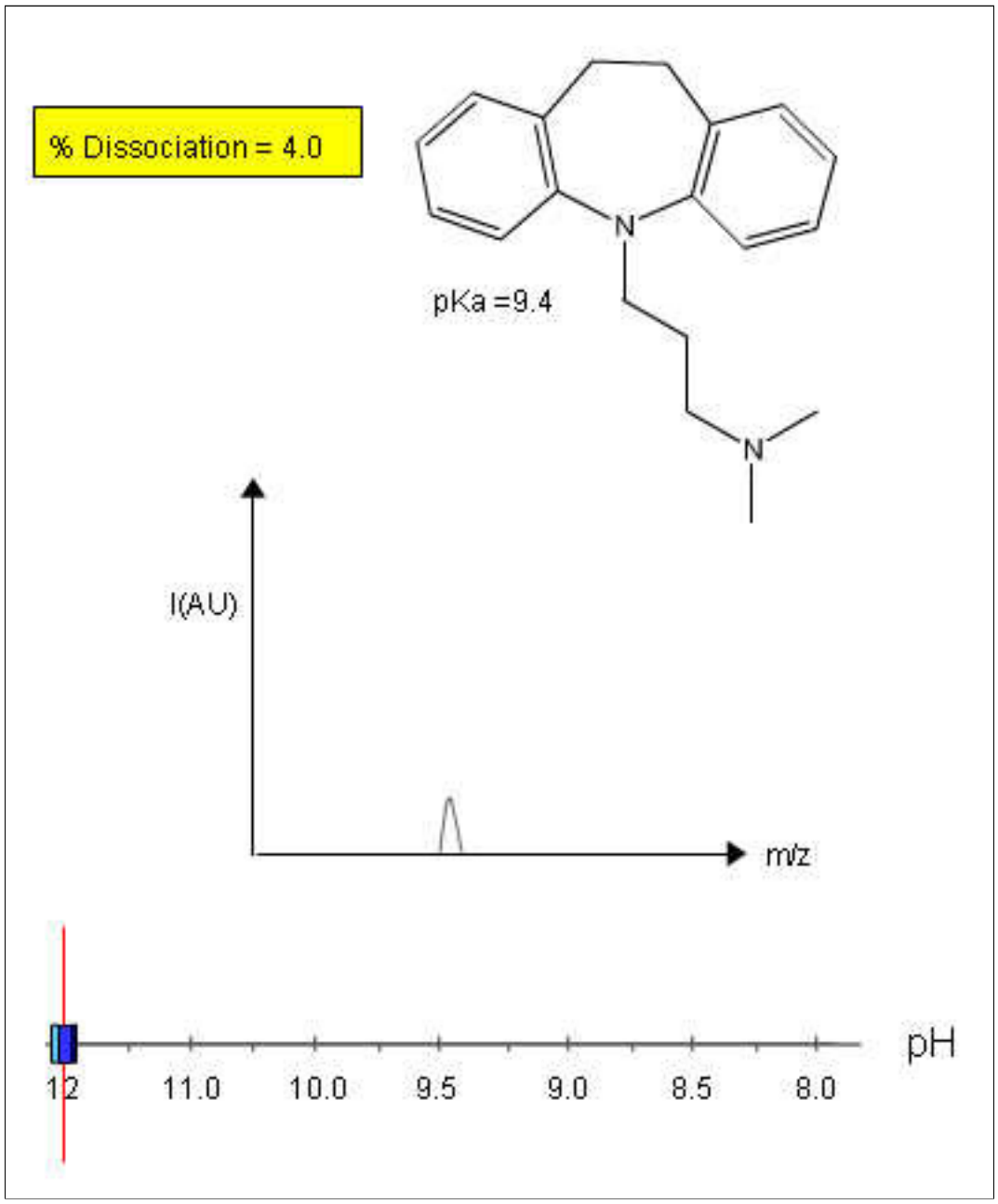


5. Although there are some general rules, you have to assume LC-MS is compound dependant.
   This means you should, where practical, perform an infusion of your compounds of interest and optimise all parameters
   Have an individual tune file for each group of compounds.
   No infusion pump? No excuse. If you're using LC-MS you need one.



6. Tune by mixing your infused sample into a representative mobile phase stream through a tee piece. This allows you to optimise source temperatures and gas flows to the proposed mobile phase flowrate and composition. Bear in mind that temperatures take time to stabilise.

7. Control of pH is critical to so many aspects of LC-MS. It is essential that your mobile phase contains components that control pH. At the very least a volatile acid (0.1% formic acid) or base (0.1% ammonium hydroxide – if the column is rated to high pH), but ideally a buffer (10mM ammonium formate), adjusted to a specified pH (2.8 or 8.2).



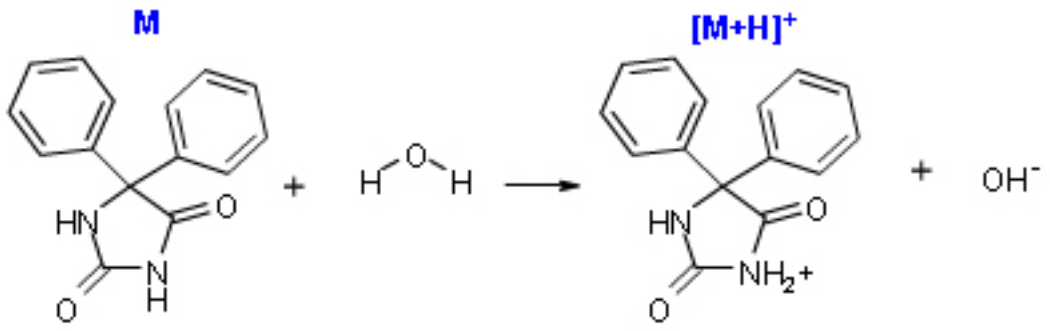
8. During tune, evaluate your compound by mixing it through a tee piece with acidic then basic pH mobile phases. It's usually worth evaluating both positive and negative ion mode at each pH.

9. Find a suitable test compound, test conditions and expected results which you know work reliably (i.e. a benchmark). Go back to this anytime you have problems with the ms to evaluate quickly if this is related to an instrument fault.

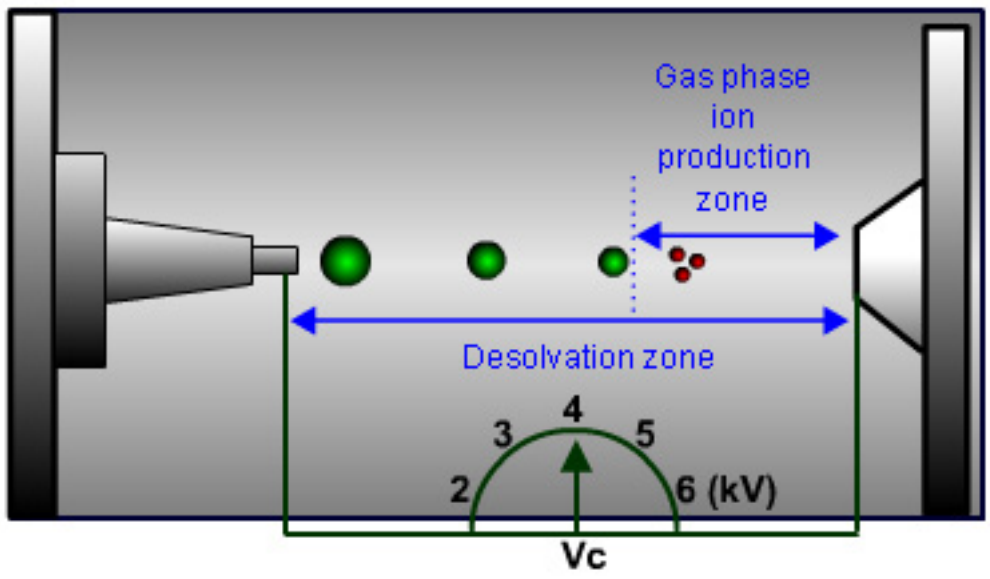
10. LC-MS still relies on a good chromatographic separation before the ms. When using SIM or SRM many quantitative problems are caused co-eluting substances entering the ion source with the analyte of interest, causing ionisation efficiency issues. It's a good idea to run full scan acquisition on a representative sample to visualise potential co-elution problems. Don't just blame the ms for quantitation issues, many of these problems are due to ineffective sample prep or chromatographic separation.

10 KEY POINTS FOR UNDERSTANDING ELECTROSPRAY IONISATION

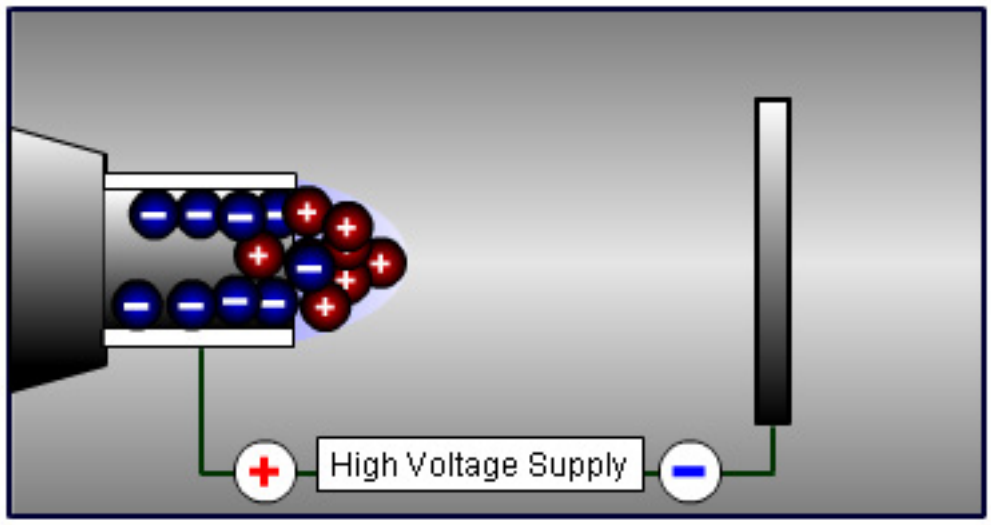
1. Eluent pH adjusted to obtain ionised forms of the analyte in solution – retention in reversed phase HPLC may need to be considered and an appropriate stationary phase chosen. The eluent flow rate is adjusted so as not to 'swamp' the source – typically <1 mL/min.



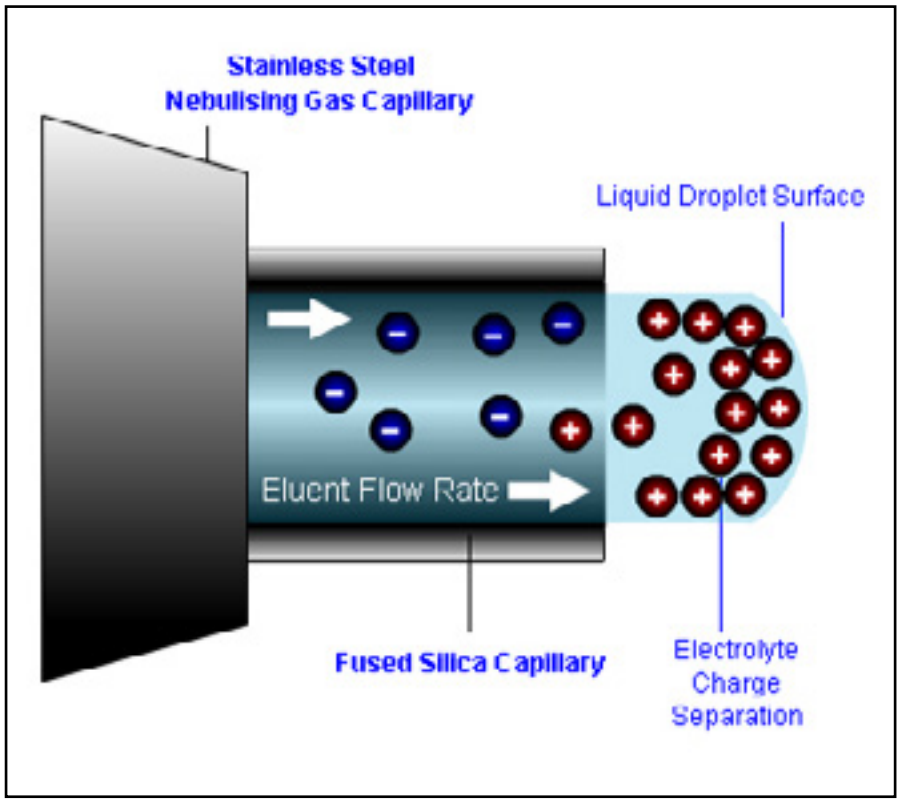
2. A voltage is applied between the capillary and sampling plate to generate an Electrospray - a special circuit which is completed by the movement of analyte ions through space (the desolvation zone).



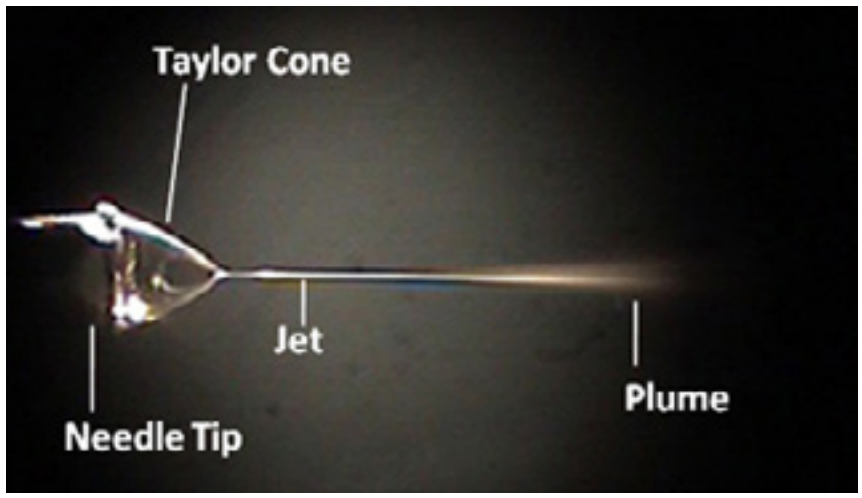
3. This applied voltage causes charge separation at the capillary tip and defines the 'mode' of spraying as an excess of +ve or –ve are created. Neutral analytes may be charged through redox, proton transfer, adduction or electrostatic processes.



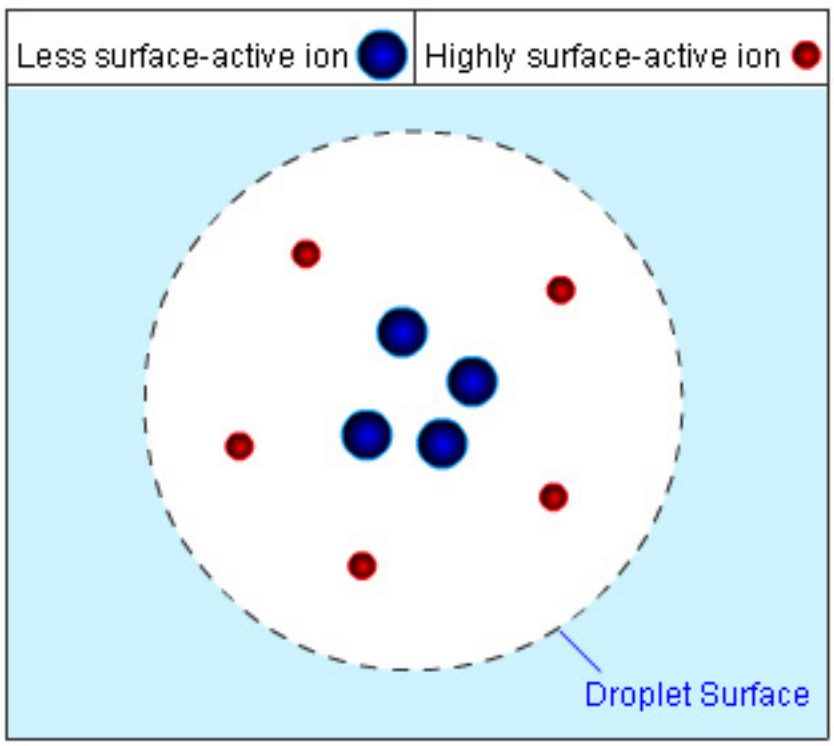
4. A droplet forms at the capillary tip containing an excess of charge and which is constrained in size by the use of a nebulising gas – this ensures the right amount of charge to droplet radius.



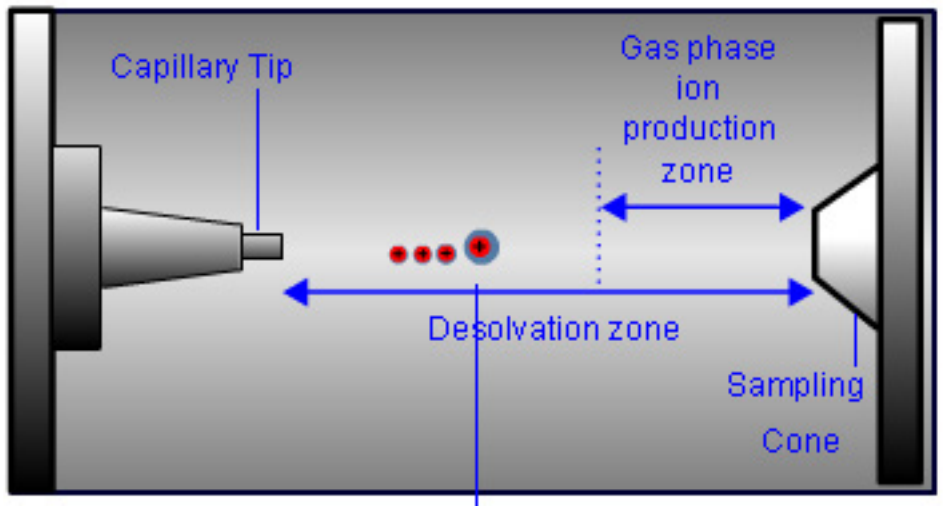
5. Droplets are sprayed from the end of a canonical cone (the Taylor cone) which forms to relive columbic repulsion at the sprayer tip once the Rayleigh limit is reached Care needs to be taken in order to apply just the right voltage to the sprayer in order to avoid 'discharge' which is an unstable spray mode.



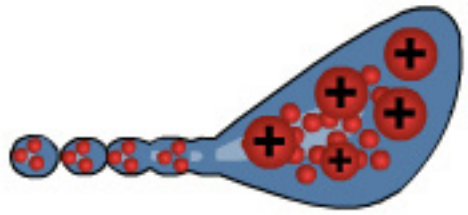
6. As the droplet travels across the desolvation zone, hydrated ions migrate from within the bulk of the droplet to the surface, from where they can ultimately be transferred to the gas phase. The radius of the ion as well as the degree of hydration will govern the ease of movement to the droplet surface – and hence its relatively intensity in the final spectrum. Small, highly charged, ions carry large spheres of hydration and are restricted in their movement from the droplet bulk to the surface. The relative ease of movement through from the droplet bulk to the surface and the competition of ions for the decreasing area at the droplet surface gives rise to ion suppression / enhancement effects.



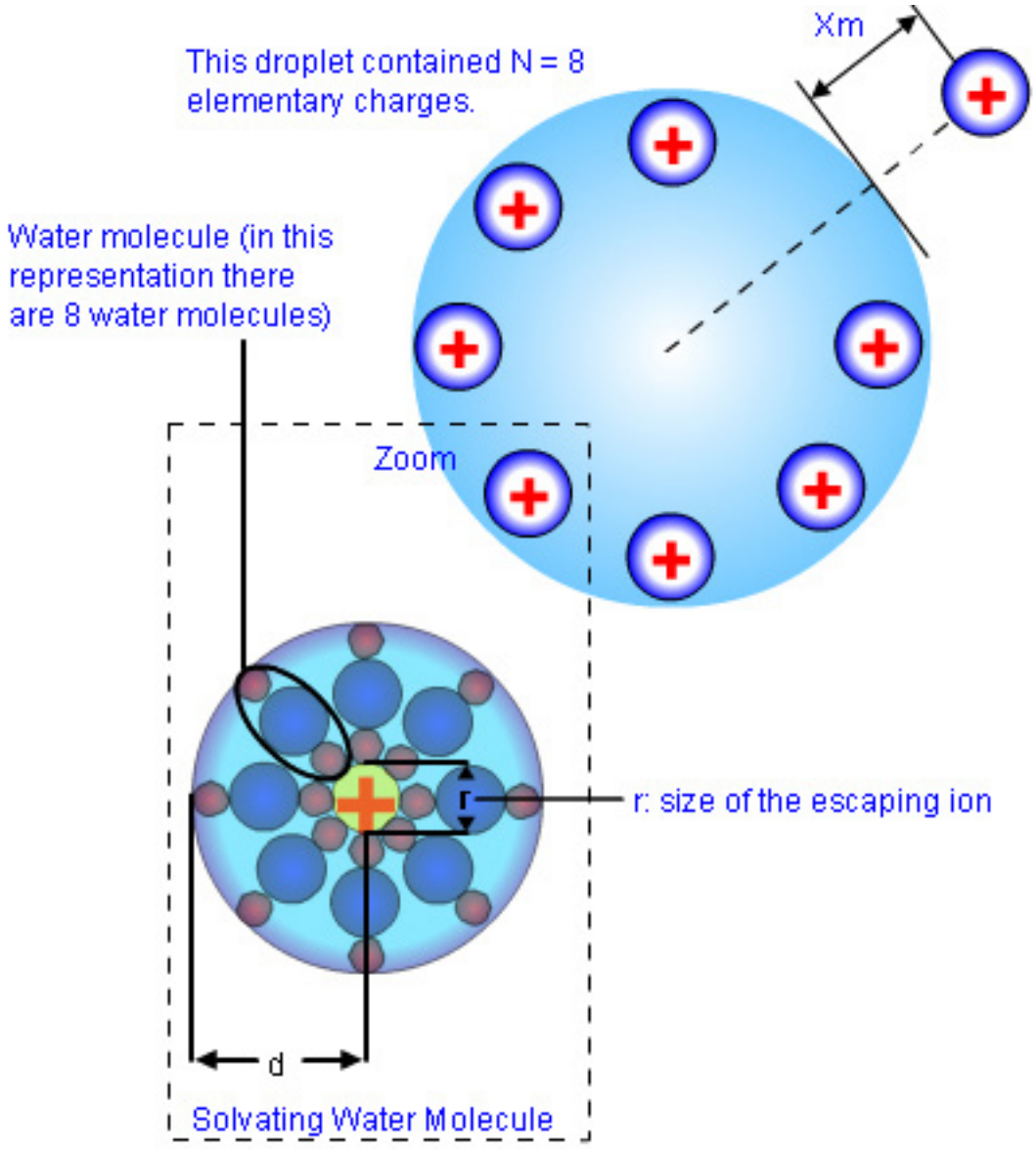
7. The droplet desolvates under the influence of a drying gas and the surface charges move together causing increasing amounts of repulsion.



8. The Rayleigh limit is exceeded and coulombic jet fission occurs – liberating a number of smaller droplets in which the charge density is much higher than in the original droplet – this then sets off a 'chain reaction' of further fissions from these smaller droplets.

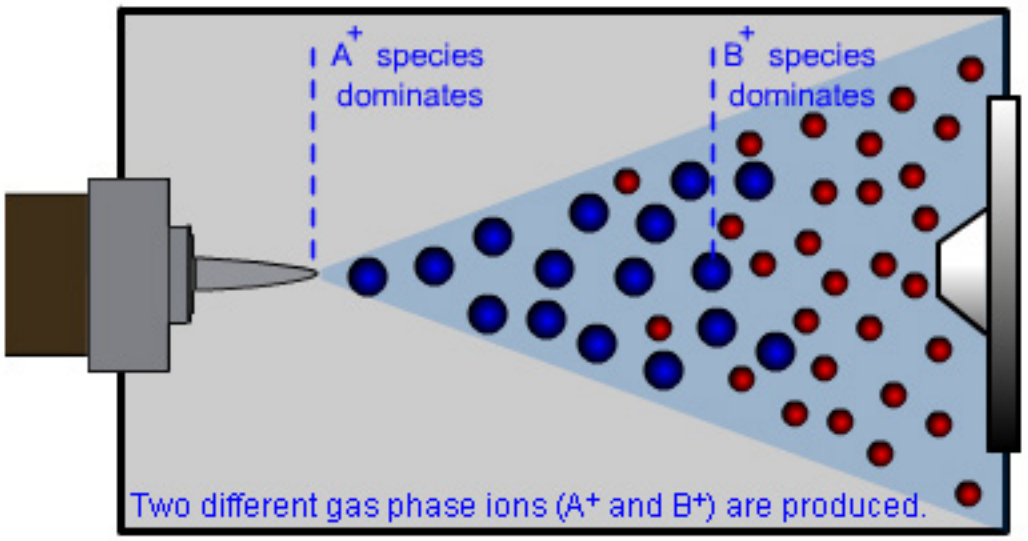


9. Once the droplet reaches a certain mass to charge ratio (or diameter) one of two things happens – a point is reached where the repulsion within the droplet it so great that it is thermodynamically favourable for a hydrated ion to be liberated from the droplet into the gas phase (the Iribarne / Thompson 'Ion Evaporation' Theory) or the droplets continue to undergo subsequent droplet fissions until they contain only a single ion which is liberated into the gas phase via further desolvation (the Dole 'Charged Residue' Theory).



10 KEY POINTS FOR UNDERSTANDING ELECTROSPRAY IONISATION

10. Once liberated into the gas phase the ions are sampled into the first vacuum region of the mass spectrometer – usually as hydrated clusters. The position of ion production within the spray, both axially and laterally, will change markedly with different analyte / sample matrix / eluent combinations which may require that the position of the sprayer relative to the sampling cone is adjusted to optimise instrument response for a particular analyte type.



MY LC-MS ISN'T BEHAVING! WHERE DO I START?

Instrument manufacturers try to convince us that mass spec is just another detector. Most of us who work with LC-MS know that's simply not the case – they can be maintenance intensive, unforgiving and generate complex information. When they're not working it can be difficult to work out exactly where the problem lies. Here's some advice to point you in the right direction :

The first step is working out if the problem is related to method / sample or if it's an instrument related fault.

1. Establish a benchmarking method - a simple, quick method that you know works 100% reliably every time. 5 replicate injections of a solution of prednisone onto a short C18 column are ideal. Generate a set of data when the instrument is working well and use this to refer to when it isn't working. Switch to the benchmarking method when you get a problem - if the benchmark works then the problem is related to something with your method / samples and not a fault with the instrument.

   If you find that the benchmark doesn't work then the problem is with your system. Your system is made of two components the HPLC bit and the MS bit. You need to work out which one of the two is at fault.

2. If the problem is related to retention times then it's nothing to do with the mass spec. The mass spec gives information on the chromatogram AFTER it's been generated by the HPLC. Any problems relating to chromatography such as wrong retention time, retention time drift and most peak shape problems are caused by the HPLC, so don't waste your time troubleshooting the mass spec.

3. Some problems can be caused by a fault with either the HPLC or the mass spec (e.g. poor repeatability, poor peak height). Prednisone is detected by both ms and UV detectors. Add a UV detector to the system and run the benchmarker. If results are poor on both UV and ms then it's an HPLC fault.

   If results are OK by UV and poor by mass spec then the problem is with the mass spec, so we'll go through some basic steps in trying to figure out what's wrong.

   Rule here is to keep things simple and move on one step at a time.

4. Using the benchmark mobile phase perform a tee-ed infusion of prednisone in full scan +ve ion 350 – 370amu, using MCA or profile acquisition. Optimise the signal using normal tuning approach.

5. If you still have no signal at this stage, use a scan range of 50-500amu and look for any masses attributable to background / bleed / noise ions. If the whole baseline looks un-naturally flat, try cleaning the source.

6. If you see a signal for prednisolone, verify that this is optimal at c.359.2amu. If it isn't, this indicates a mass accuracy problem so the ms should be recalibrated.

7. If you see the signal at 359.2 inspect the shape of the mass peaks. If they look unusually tailed or fronted or show inadequate resolution try re-tuning using the advanced parameters of your instrument. If this doesn't resolve the problem, it's time to vent and clean.

8. If the mass looks accurate, the mass peaks appear to be the correct shape, but it's just that sensitivity is low then it's time to clean the source.

9. Cleaning the source. If you're going to all the trouble of venting the mass spec, don't do half a job of cleaning the source. Take an extra 15mins to strip the instrument back as far as your manufacturers recommend – this should be almost back to the mass analyser i.e. the complete ion source and focusing lenses. Don't skimp on the cleaning – take your time do it fully and correctly. Cut any corners here and it just means you'll have to repeat the next day.

   If you're not comfortable doing this then it's time you learned. Either read the manual, ask your service engineer to demonstrate it or get booked onto a training course. If you operate an LC-MS you need to be comfortable and competent at stripping and cleaning the source.

10. If you've done all this and the problem persists, then next step is have a chat with your service engineer. Many mass specs have software diagnostics that can allow an engineer to diagnose a problem over the telephone. Always try and chat to the engineer before booking them to visit – it may be something simple that you can fix yourself.

10(ISH) WAYS TO PREVENT LC-MS CONTAMINATION

1. **Clean, particle free solvents**

   Particulates can cause havoc in an LC-MS system by blocking components, while contaminants, such as alkali ions, plasticizers, and surfactants interfere strongly with LC-MS by causing higher background noise and the formation of adduct ions. Non-volatile components will result in a contaminated ion source which needs cleaning more often.

   Therefore, LC-MS grade solvents should always be used for LC-MS applications. LC-MS grade solvents will be pre-filtered by the manufacturer with a 0.2 µm (or smaller) filter. Further filtration may not be required and could introduce contamination from the filter itself (Figure 1).

   These solvents will also meet the stringent requirements for low levels of impurities (e.g. metal ions).

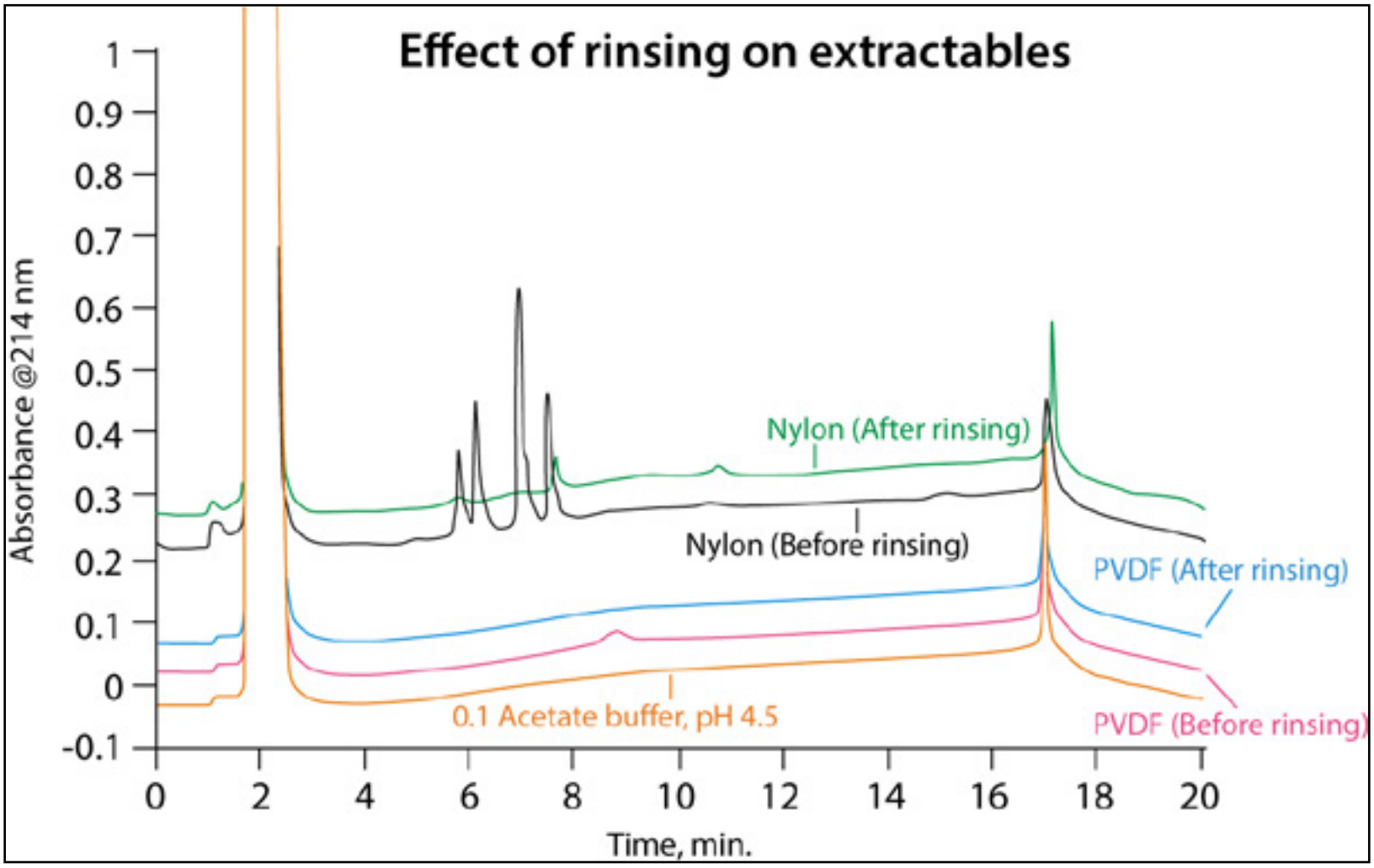


Figure 1: Effect of rinsing to remove extractables from syringe filters.[1]

2. Use ultra-pure water

Water should never be overlooked as a possible contamination source. The water used in the lab serves a myriad of purposes from washing glassware, to making up standards and blanks, to being a component in the mobile phase. Impurities in water can collect on the column during equilibration with the weak solvent, which could cause damage to the column and affect chromatographic results (Table 1). Therefore, ultra-pure water should be utilized for LC-MS applications.

Ultra-pure water is particle free, chemically clean, and has a resistivity of 18 mΩ. Purification systems for ultra-pure water use reverse osmosis to remove most contaminants, ion exchange to remove ions, carbon filtration for the removal of organics, UV sterilization to kill bacteria, and a pharmaceutical grade 0.2 µm membrane filter to remove particulates.

Contaminants	Effects
Organics	Noisy or drifting baselines Ghost peaks Extensive contamination can result in shifting retention times and distorted peak shapes Excess background ions in MS
Ions	Some ions absorb in the UV range (e.g. nitrites and nitrates) Metal ions can form adduct peaks in MS detection
Particles	Damage HPLC pump and detector Increase system back pressure
Bacteria	Behaves as particulates (increased back pressure) By-products include organics and ions

Table 1: Contaminants and their effect on HPLC(-MS) systems.

3. **Prevent microbial growth**

Microbial growth can be particularly problematic for UHPLC systems which can be much more sensitive to blockages due to the smaller tubing diameters and column frit porosities. Aqueous mobile phases and water are prone to microbial growth (even over short time periods); this can cause extra peaks in gradient elution and increase background absorbance during isocratic methods. Microbial growth behaves as a particulate and can block filters, frits, and columns, as well as causing check valve malfunctions. All of these problems will result in high pressure which can damage columns and cause system shutdown.

Microbial growth can be prevented by preparing mobile phases fresh each day, filtering, and degassing. To avoid microbial growth when the system is idle, flush all buffer components from the system and column and store both in an appropriate solvent e.g. 60:40 organic:H2O (Figure 2).

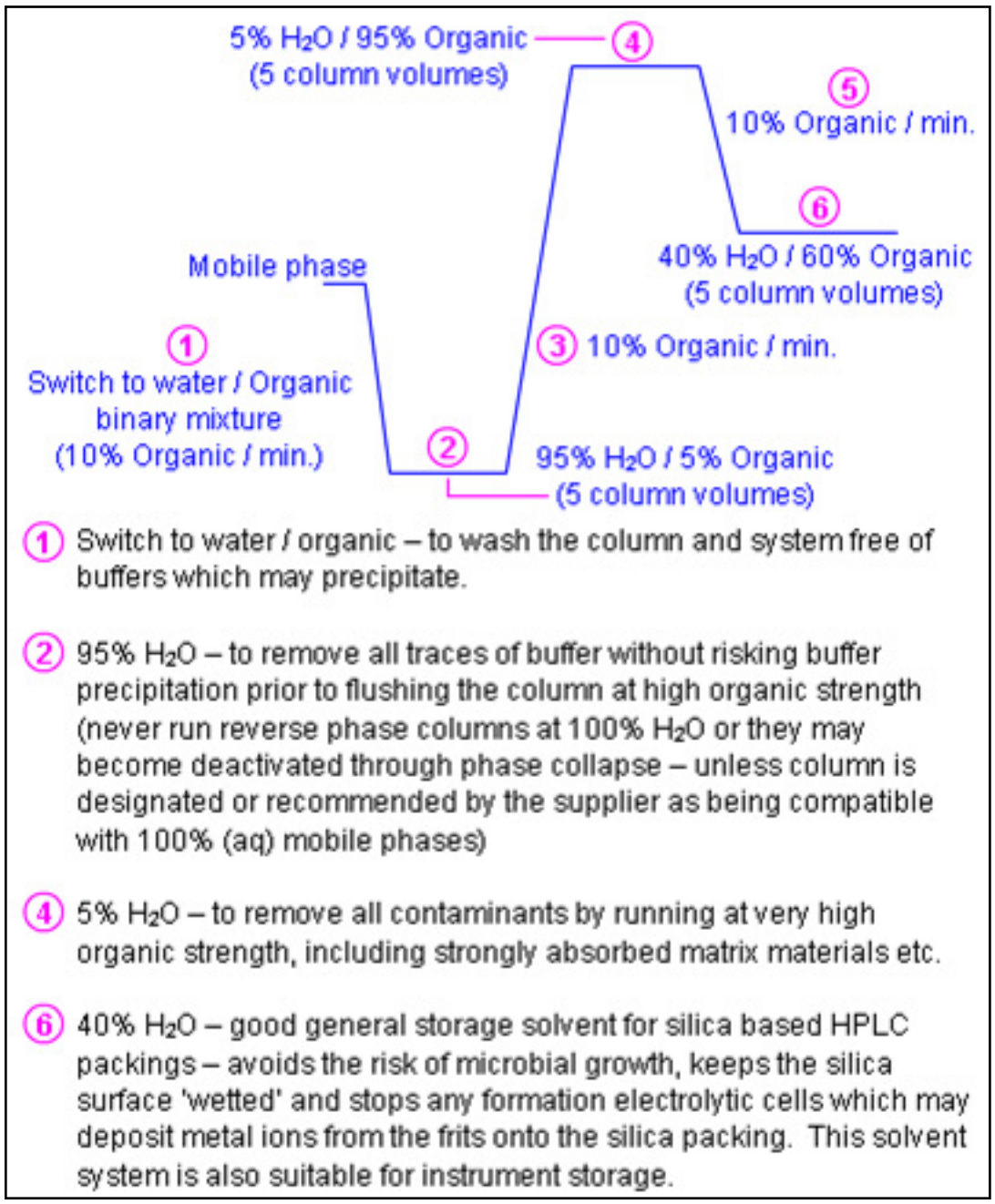


Figure 2: HPLC system and column flushing gradient.

4. **Degas all solvents**

Degassed mobile phases will produce steadier baselines and there will be reduced risk of forming bubbles in the system which can adversely affect chromatography (flow rate problems, retention time issues). Even if a system has an inline degasser it is recommended that solvents are degassed prior to use. The optimum method for degassing solvents is vacuum degassing (Figure 3).

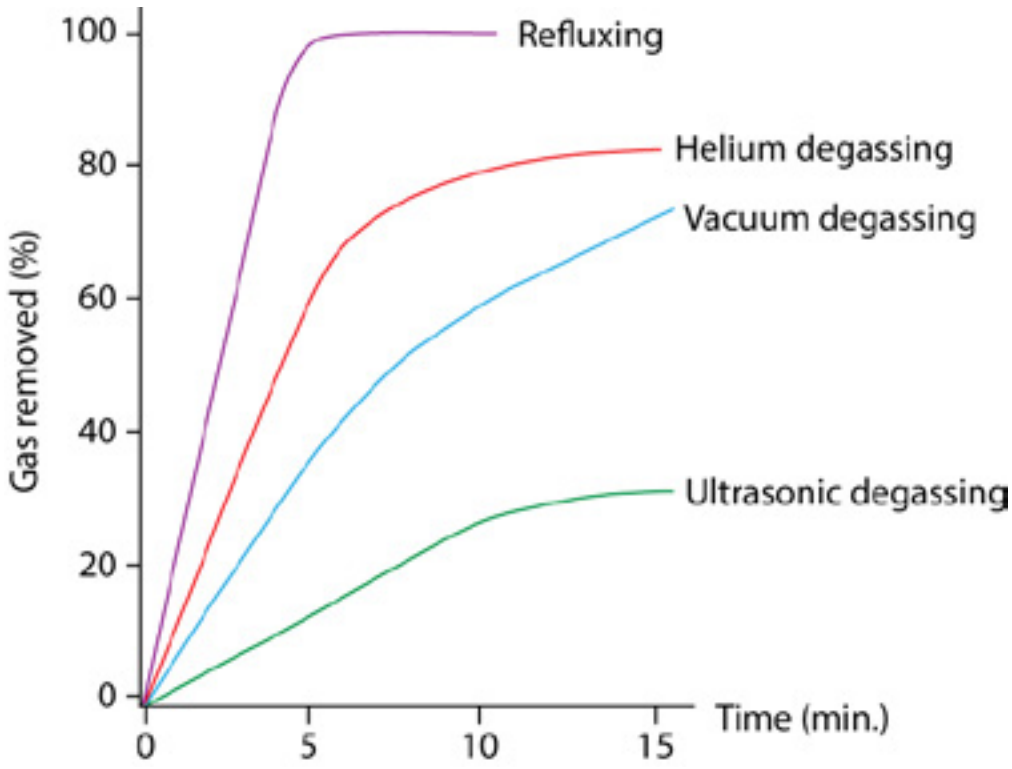


Figure 3: Solvent degassing methods.

5. **Minimize the use of additives**

Use the lowest amount of the required additive possible to reduce background noise. Any additives which are being used should be volatile to avoid contamination of the ion source (i.e. use formate or acetate buffers and not phosphate, Table 2). Some additives cause signal suppression, for example TFA; formic acid can be a good alternative if sensitivity cannot be sacrificed. Furthermore, all additives should be of the highest possible purity, i.e. low concentration of metal ions (Figure 4).
Remember, if a little bit works, a little bit less probably works better - 10 mM or 0.05% v/v is a good place to start.

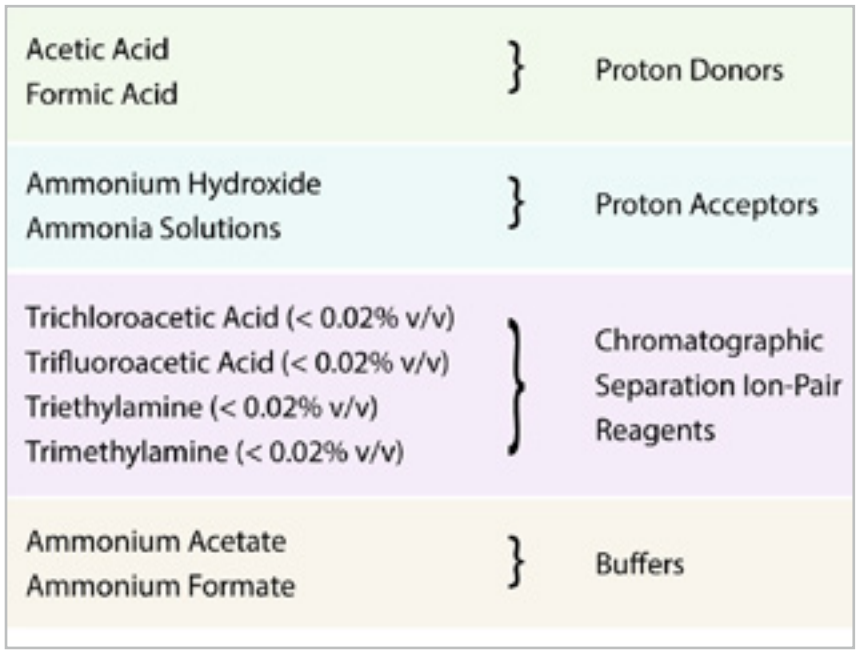


Figure 4: Acids, bases, and buffers suitable for LC-MS applications.

Buffer	pK_a	Buffer Range	Formula	Buffering Equilibrium	10 mM Concentration Mobile Phase Preparation*	pH Adjustment (Acid or Base)
Ammonium acetate pKa1	4.76	3.8-5.8	CH_3COONH_4	CH_3COOH <--> CH_3COO^-	0.77 g	CH_3COOH or NH_4OH
Ammonium acetate pKa2	9.20	8.2-10.2	CH_3COONH_4	NH_4^+ <--> NH_3	0.77 g	CH_3COOH or NH_4OH
Ammonium formate pKa1	3.80	2.8-4.8	NH_4COOH	$HCOOH$ <--> $HCOO^-$	0.64 g	$HCOOH$ or NH_4OH
Ammonium formate pKa2	9.20	8.2-10.2	NH_4COOH	NH_4^+ <--> NH_3	0.64 g	$HCOOH$ or NH_4OH

Table 2: Properties of acetate and formate buffers for LC-MS. * Addition of volume or mass per 1 liter.

6. **Proper solvent storage**

Although it is ideal to make up eluents fresh each day to avoid microbial growth, realistically solvents will sometimes be stored. The proper length of time for solvent storage is a much disputed subject in the literature; however, the information below can serve as a good initial guideline. Each method should be monitored and if chromatography starts to deteriorate then solvent storage limits can be reassessed.

Deionized water - The most conservative value is 3 days. With most people replacing after 1 week.
For aqueous/organic solutions (without buffer) - 3 days is a conservative time frame. With one week to one month being the average.
Buffer solutions - 3 days. Although with UHPLC 1 day has been noted. Also, microbial growth has been shown to alter ion chromatography.
Aqueous solutions < 15% organic - 1 month.
Aqueous solutions > 15% organic - 3 months. Addition of 20 - 30% organic inhibits microbial growth extending shelf life. Evaporation of more volatile solvents over longer periods should also be kept in mind.
For pure organic solvents - these should be stable for extended periods of time and normally the manufacturer will give a use by date (if this is not on the bottle then please contact your supplier for this information). Care should be when using solvents that could produce peroxides (ethers, THF) as these may have shorter shelf lives if exposed to air or other oxidizing components.
For mixtures of organic solvents - The danger here is from selective evaporation of one or other of the solvents which will change the composition of the eluent over time and in turn will affect chromatography. Even if the bottle is well sealed there may be some change in composition and shelf life of 1-3 months is common practice. Even capped bottles may suffer from some evaporation.

Most labs will find that they make up eluent frequently as you are limited to the volume that can be stored, and therefore, if you are running your HPLC instrument everyday stocks will be rapidly depleted.

Solvents should be stored in clean glass reservoirs with covers to prevent airborne contamination. Reservoirs should be glass as plastic can promote plasticizer contamination (e.g. phthalates).
Although it should be noted that sodium contamination can originate from glassware. See this article for more information Controlling Na and K Adducts in LC-MS »

Also remember not to prop up bottles to get last drop out. Apart from the risk of running the pump and column dry, mobile phases evaporate from the surface; therefore the mobile phase at the top of the bottle will have changed composition from the bulk. This portion from the top is exactly what will be running through the column if you use the last drops in the bottle.
Do not top off solvents as the composition may not be correct due to evaporation from the original solvent; instead discard the old solvent, rinse the bottle and filters with new solvent and refill with fresh mobile phase.

7. **Correct cleaning of laboratory glassware**

   Soaps and detergents can play mayhem with an LC-MS system, causing ion source contamination and high background noise. Therefore, avoid using soaps and detergents to wash glassware which is to be used for LC-MS. Glassware should not be washed in dishwashers which contain detergent contamination. Glassware should be cleaned by rinsing with organic solvent then water, and then rinsed with the solvent which will be used in the piece of glassware.

   For more aggressive cleaning glassware can be sonicated with 10% formic or nitric acid, then water, then methanol or acetonitrile, and finally water. This process can be repeated.

   Glassware which contains microbial growth can be treated using an autoclave and all filters and tubing between the mobile phase reservoir and instrument should be replaced. The instrument itself can be rinsed with acetonitrile or methanol and left to sit overnight.

8. **Make sure samples are clean**

   Filter, filter, filter. Make sure all samples are filtered prior to injection to avoid blockages in tubing and column end frits. Make sure to select the correct filter membrane (i.e. filter material, size, and porosity). The porosity of the syringe filter should be considered in conjunction with the porosity of the column inlet frit and/or packing material diameter so that any particulates which would be large enough to block the column inlet frit, or column itself, are removed prior to reaching the HPLC system. For example if a column is packed with particles which are less than 2 µm a 0.2 µm UHPLC filter should be used. Syringe filters with a 0.2 or 0.45 µm porosity are suitable for the filtration of samples which will be analyzed using columns packed with particles > 2 µm.

   Filtration is important, but only gets rid of particulates. The real bad guys are all the materials that dissolve but are non-volatile. Sample matrix is probably the biggest source of contamination. For samples which contain high levels of non-volatile matrix components (i.e. salts in biological samples) consider more rigorous sample preparation techniques, such as solid phase extraction - as well as reducing contamination of the LC-MS system removal of these matrix components will reduce background noise and interferences which can improve analytical results.

   Any plasticware which is used (e.g. pipette tips, well plates, vials etc.) should be high quality, phthalate free to avoid contamination.

9. **Use clean fittings and tubing**

   Any tubing and fittings which come into contact with the sample or mobile phase (e.g. all flow path components - check valves, seals, o-rings, filters etc.) should be as inert as possible, compatible with any components or solvents being used in the mobile phase, and be of high quality. Tubing made of polymers can be made of polymers which may contain plasticizers which may leach into the LC-MS system.

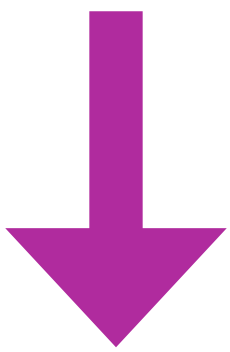
10. Wear gloves

There is nothing worse than analyzing a complex mass spectrum and realizing the peaks are keratin (human protein from skin cells, Table 3). MALDI is particularly sensitive to keratin contamination. Or trying to interpret a spectrum which contains a plethora of sodium adducts which can come from handling samples, labware, and instrument parts without wearing gloves.

897.4140	1179.6010	1365.6399	1838.9149
973.5318	1184.5911	1373.6549	1993.9772
1037.5267	1193.6166	1383.6909	2312.1482
1060.5639	1234.6769	1434.7705	2383.9524
1066.4992	1307.6782	1474.7858	2510.1323
1066.5169	1320.5834	1699.8251	2705.1617
1140.5649	1357.7188	1707.7727	2831.1947
1165.5853	1357.6963	1716.8517	3312.3087

Table 3: Common keratin peaks.[2]

BONUS TIPS... KEEP READING



11. **Look after columns**

Many of the points above will help to keep columns contamination free. However, some contamination may be unavoidable due to the type of analytes being analyzed; for example proteins can precipitate and get trapped at the head of the column or organic contaminants which are difficult to remove using sample preparation may also get trapped. Column contamination will reduce column lifetime, can alter chromatographic results, can increase background noise, and cause system pressure issues.

To help remove contaminants run a high organic wash at the end of the analytical method. If columns become badly contaminated follow manufacturer's cleaning guides to help restore them. If this information is not available then generic cleaning methods can be found at: HPLC Column Cleaning and Regeneration »

Be careful with column chemistries. Some types bleed more or give higher background signals than others (e.g. some polar embedded phases).

12. **Flow rates and splitting**

Contamination can be reduced significantly by using a post-column diversion valve which is automatically set to run to waste at all times except for the retention time period containing the peaks of interest. Early elution (unretained material in the t0 peak) and late eluters at the tail of gradient are the real culprits for clogging sources.

Make sure the temperatures and gas flows in the source are suitable for the mobile phase aqueous content and flowrate. If the temperature is too low, the liquid condenses in the source.

For electrospray, keep the flow low. Use a post column splitter to reduce flow or use a 2.1 mm column with a flow rate of 0.3 mL/min.

13. **Tune**

Optimize/tune MS conditions to get the best sensitivity for the compounds of interest, then use the high sensitivity to reduce the amount of sample injected. It is not just matrix which contaminates - large amounts of actives cause problems too.

CONTROLLING NA AND K ADDUCTS IN LC-MS

Adduct ions are prevalent in LC-MS analyses and can come from any number of sources. An adduct ion is any ion formed by adduction of an ionic species to a molecule, and can be present under all modes of ionization (e.g. ESI, APCI etc.) and under different ionization polarities (positive or negative ionization).

The commonly observed protonated molecule, [M+H]+ is technically an adduct ion. However, adduct ions which originate from alkali metals, solvents, or other metal species can cause problems when identifying the molecular ion and interpreting mass spectra (Figure 1, Tables 1 and 2).

Adduct ions in APCI must be volatile which means ammonium, chloride, and water adducts can occur but metal adduct ions cannot.

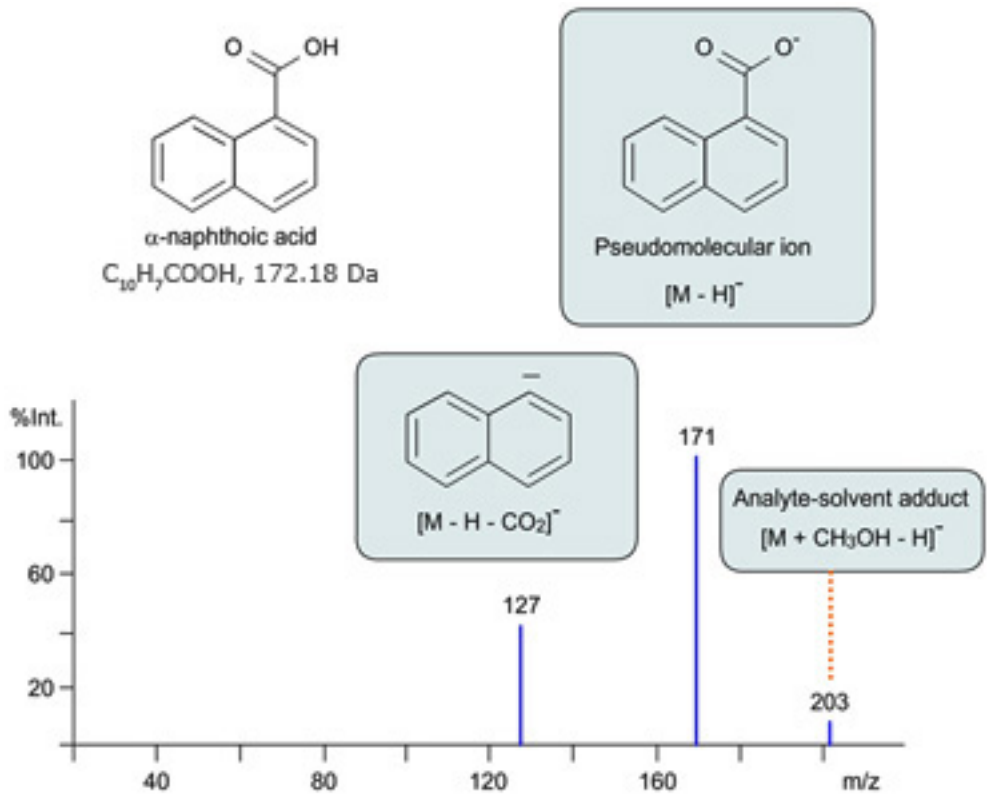


Figure 1: Negative ESI spectrum of α-naphthoic acid.

Observed	Explanation	Mass
$[M-H]^-$	Deprotonation	M-1
$[M-H-nH_2O]^-$	Deprotonation and loss of H_2O	M-1-(nx18)
$[M+Cl]^-$	Ion attachment	M+35 (37)
$[M-2H+Na]^-$	M + Na adduct	M+21
$[M-H-CO_2]^-$	Carbon dioxide loss	M+45

Table 1: Typical adduct ions encountered in ESI negative ion mode.

Observed	Explanation	Mass
$[M+H]^+$	Protonation	M+1
$[M+NH_4]^+$	Mainly when using CH3NH4	M+18
$[M+H+nH_2O]^+$	Water cluster	M+1+(nx18)
$[M+H+H_2O]^+$	M + H_2O adduct	M+19
$[M+Na]^+$	M + sodium adduct	M+23
$[M+K]^+$	M + potassium adduct	M+39
$[2M+H]^+$	Analyte dimerization	(2xM)+1
$[M+H+CH_3CN]^+$	In presence of CH_3CN	M+42
$[M+H+CH_3CN+nH_2O]^+$	Water-acetonitrile cluster	M+42+(nx18)

Table 2: Typical adduct ions encountered in ESI positive ion mode.

CONTROLLING NA AND K ADDUCTS IN LC-MS

The sources of adduct ions are prolific, but an understanding of these sources can help to eliminate or reduce the occurrence of adduct ions. Sodium and potassium adducts are some of the most common LC-MS adducts produced. Sodium adducts will appear 22 m/z units above the protonated molecule and potassium adducts will be 38 m/z units above (Figure 2).

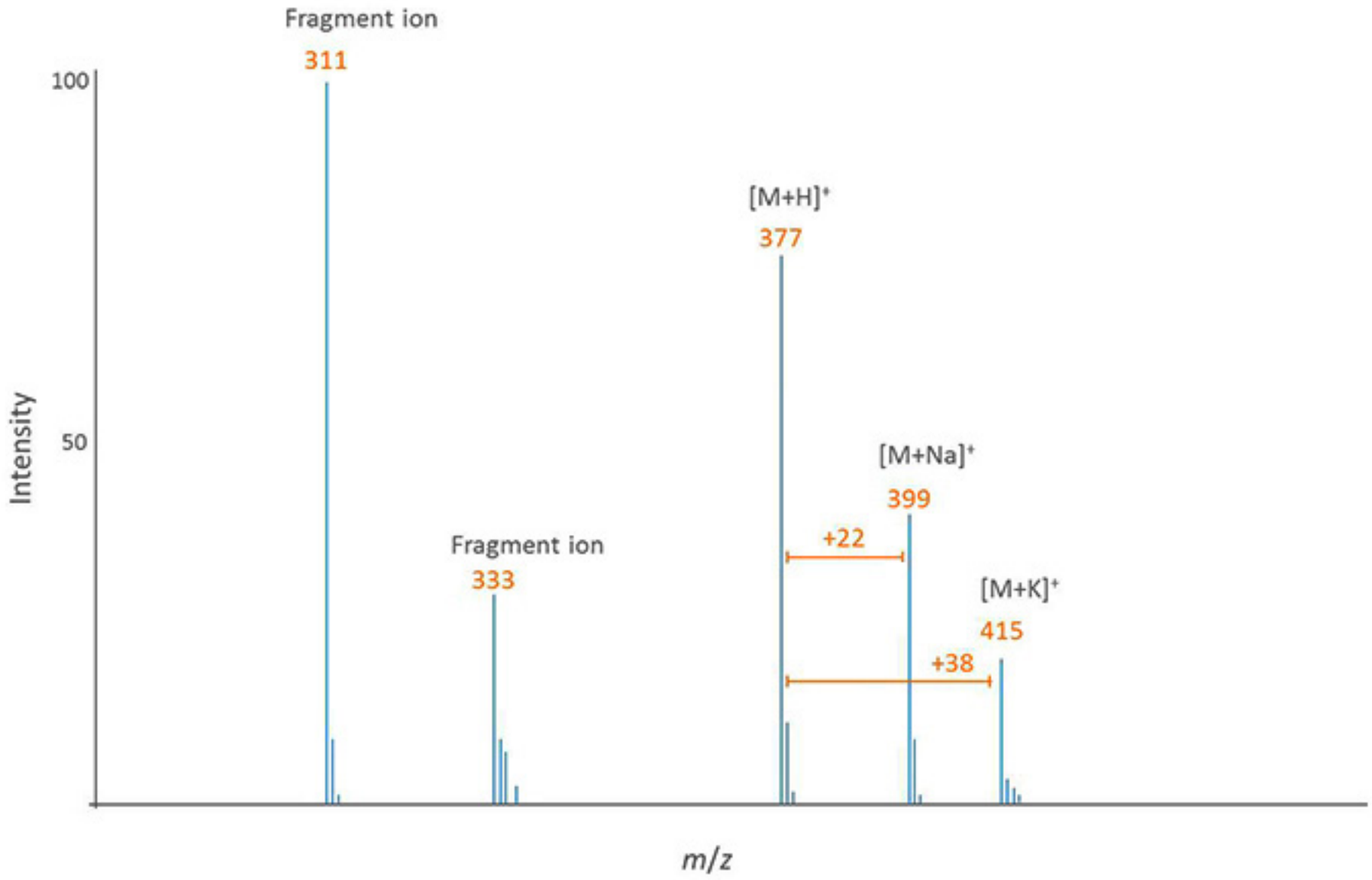


Figure 2: Mass spectrum illustrating sodium and potassium adduct ions to an analyte with a molecular mass of 376.

CONTROLLING NA AND K ADDUCTS IN LC-MS

The most common source of these species is from glass due to the salts used in the manufacturing process. If excess adduct formation is seen due to the use of laboratory glassware consider switching to MS certified glass or high quality plasticware. However, the use of plastic comes with its own problems due to plasticizer contamination (e.g. phthalates).

The use of high quality phthalate free plastic can reduce this type of contamination, and as the m/z values will be at fixed values they may be easier to discount during spectral interpretation - unlike adduct ions which will have varying m/z values based on the analyte's mass. Biological samples can exhibit pronounced levels of alkali metal adduction as there is a high endogenous concentration of various salts, with others being added during sample preparation.

Sample clean up processes, such as solid phase extraction, are effective in remove matrix compounds from biological samples, which will have the added benefit of reducing possible ion suppression. Mobile phase design is important for all LC-MS applications, in particular the use of volatile components to avoid contamination of the ion source. Avoiding sodium- and potassium-based pH and ion-pair reagents will help to reduce the presence of alkali metal adducts. Handling samples and labware without wearing gloves can also transfer enough sodium to produce significant adduct ions.

In some cases, the protonated molecule may be completely absent with only metal adduct species present. There are two strategies which can be used to deal with alkali metal adducts.

- Lower the pH - this is the preferred method for dealing with unwanted alkali metal adducts. An organic acid, such as formic acid, is added to provide an excess of protons relative to metal ions which drives all or a major portion of the ion formation to the protonated molecule [M+H]+. Additionally, lowering the mobile phase pH should improve ionization efficiency providing better limits of detection for the protonated molecule.

- Add potassium or sodium acetate to the mobile phase - this approach can be used if lowering the pH does not eliminate the majority of the metal adduct species. A large excess of metal ion produces nearly exclusive formation of the metal adduct ion species. The reagents employed must be volatile so as to avoid precipitation of involatile salts in the interface which can cause blockages and contamination.

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