



# Top 10 Tips Solid Phase Extraction

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sciences

## 1. Know Your Compounds!

Properties of the compounds being analysed can have a big impact on which type of SPE can be performed (check out our handy SPE guide on [www.microplates.com/spe-guide](http://www.microplates.com/spe-guide)). Two of the most important values are the **pKa** and **Log P** of your analytes of interest:

**pKa** = The point at which the analyte is half ionised and half un-ionised.

**Log P** = The measure of hydrophobicity of a compound.

## 2. Optimise Loading pH

pH can have a large impact on any SPE method and can be used to optimise retention of compounds on an SPE product.

For example, reversed phase method adjust pH to neutralise any charge on an analyte.

This will make them more hydrophobic improve interactions more with the resin. For acidic compounds, adjust 2 pH units **BELOW** pKa and for basic compounds, adjust 2 pH **ABOVE** pKa.

## 3. Refine Wash and Elution Strength

When using a mixed mode or reversed phase SPE product, wash and elution strength can be tweaked to give the cleanest extracts. Log P is a good property to help decide on what to set these at.

**High Log P** - Use stronger organic strengths in the wash steps.

**Low Log P** - Reduce the organic strength of the elution solvent to inhibit hydrophobic interferences whilst still eluting the analyte of choice.

## 4. Fresh Standards Are Best!

Some analytes are susceptible to degradation by heat and light and can precipitate out of solution when stored for long periods of time. This will impact the calibration standards and recovery values of a run. Start your procedure strong with freshly made standards before you start.

## 5. Know Your Sorbent!

Find out the pKa of the sorbent you are working with and use this with your analytes pKa to adjust pH of solutions on load and wash steps without deionising compounds and resins (weak ionic resins only).

## 6. Sorbent Conditioning

Now that you know the pKa of your sorbent (Step 5), your sorbent bed can be activated and ready to bind analytes by conditioning. In most silica-based SPE sorbents, the silica is bonded with a functionalised moiety that is typically lying flat on the surface of the particles.

The conditioning step (using a water-miscible organic solvent e.g. MeOH) causes these bonded functionalities to 'stand up' on top of the particles, giving them the most surface area for analytes of interest to interact/bind to. If the sorbent is water-wettable, then this step may not be needed but it can increase cleanliness of extracts by performing a solvent wash of the sorbent bed.

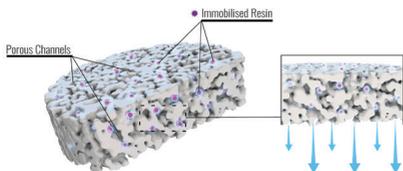
## 7. Go With The Flow (Rates)

Flow rates are important for a successful reversed phase SPE workflow. The retention mechanism relies heavily on Van der Waals forces of interaction between the sorbent and analytes of interest. Fast flow rates during a SPE procedure reduces the ability of the analyte of interest to bind properly to the sorbent media and can result in breakthrough on load and wash steps of an SPE method. Slow and steady flow of samples wins the race. Aim for a flow rate that is less than or equal to 1 drop a second (~ 1 ml /minute).

## 8. Loose-Packed vs Composited

Loose-packed material can cause a number of problems to the sorbent bed, including channelling, compression, voiding and inconsistencies in bed weight to bed height ratio differences due to lack of compression of the top frit after loose filling (Figure 1.). All of these factors can negatively influence recovery and reproducibility of testing. Consider composited products like the Microlute<sup>®</sup> CP range which have been designed to eliminate these issues and improve both recovery and reproducibility of SPE methods.

Porous structure with interconnected network of channels containing immobilised chromatographic resin.



Loose-packed mixture of resin and base material.

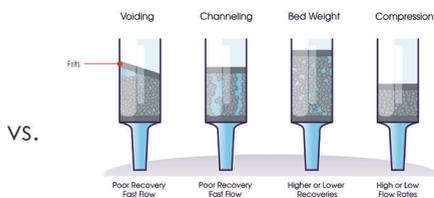


Figure 1. Microlute<sup>™</sup> CP, the composited sorbent vs. traditional loose-packed SPE

## 9. Dry The SPE Resin Before Elution

Silica is very susceptible to stationary phase collapse when dry. Do not let silica-based resins dry out at any point before completing the final wash step of the SPE procedure – this is especially true during the load step. This prevents reduced recoveries by limiting the possible interactions between analytes and bonded phase.

*The following tips applies to both polymeric and silica resins:*

- After performing the wash step(s), make sure to dry the resin using vacuum or positive pressure until the resin is completely dry (we recommend applying positive pressure/vacuum for at least 3 minutes) or until no wash solvent can be seen.
- If your method calls for a dry down and reconstitution step, drying your resin significantly decreases the dry down time, water is present in most wash solvents, which is much harder to evaporate than most organic solvents.
- If your method doesn't call for a dry down step, not drying the SPE resin after the wash step can cause for the final solution for injection to be less concentrated, causing reductions in sensitivity.

## 10. Optimal Dry Down

Ensure your dry down method is vigorous enough to visibly disturb the surface of the elution solvent, but not so vigorous to cause the possibility of expelling the solution out of the wells as this will cause severe %RSD and recovery differences. This can be quite difficult as the level of solvent will move during dry down.

To overcome this, use evaporators that can be programmed or automatically adjust for efficient solvent evaporation such as the Porvair Sciences Ultravap® range of evaporators which allows dry down to be optimised throughout all stages of the process without any risk of sample contamination.



### Technical Support

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