Overview of SPE Product Descriptions and Rationale of Design

Since the SPE industry generally just gives the mass of packing and the volume of the tube, with an occasional upper limit for the most strongly retained species (For example: one company listed 5mg capacity for a 10mg C18 column, this begs the question "5mg of what?"). It has taken time to coordinate our literature and labels as my thinking has evolved. Any discrepancy is because I had been conflicted about how to list these products.

Of the many iterations listed, I concluded that in addition to describing the physical composition of the tubes (mass and volume) it would be best to list a range for the **mass and volume loading** for samples. If doing protein digest desalting samples contain polar and non-polar components, so listing these parameters would be more useful than just how much packing material is in the tubes. This is so, as a first approximation, one would not have to do loading experiments to actually determine SPE loading for a particular sample (See **Solid Phase Extraction:** Six-Step Method Development) for the complexity of establishing a validated method.

Furthermore, most are more worried about recovery of lower amounts of sample rather than higher amounts. So a listing of 17-170μg represents an estimated range for the **upper capacity** for a 50mg C18 (MIDI) column. This is derived from HPLC loading recommendations for (preparative) **elution** chromatography of ~0.2mg/g - 2mg/g, which SPE is not (SPE is a bulk binding experiment.). Since these are HPLC grade packing materials, recoveries should be comparable to those of HPLC columns (which contain stainless steel frits which can contribute to sample losses of phosphate compounds, for example), so maybe *ca*. 95-99% is a reasonable estimate since SPE doesn't have ss frits? Having all this information makes it ripe for confusion. I hope you can agree on my choices. If not, I am open to suggestions.

Regardless, these numbers are for the maximum capacity and your concern might be for **minimum mass capacity**. Thus here are the issues with small samples which need to be discussed:

- First there is the volume of the load. Isocratic elution can occur with too large a volume. Thus extra volume of packing is your friend (if the recoveries are near quantitative) since it allows a larger loading volume and rinse volume. The data on my site from Brian Hampton (Effect of excessive loading volume on SPE Tips or Trap Columns) shows that after 7 bed volumes of load and rinse, one begins to lose the weakly retained species. Actually, the volume for this is one chromatographic void volume less than the k' for the elution of that aspect of the sample. Since we don't have HPLC data in advance, I made my recommendation as 2 bed (i.e. chromatographic void volumes) of load and rinse as a conservative guide. If you have better elution volume (k') information about your sample, then use that for loading and rinse volumes.
- Next there is the linear velocity consideration for slowly diffusing compounds (i.e. peptides). The columns can have a large aspect ratio (narrow / long) or (wide / short)

like the BioPureSPN. So for 100μL of liquid volume the linear velocity in the narrow / long will be substantially greater than for the wide / short, BioPureSPN. The caveat is that one needs to orient the BioPureSPN the same way each time to assure the same volume of rinse and elution is applied when the columns are in a fixed angle centrifuge. Recoveries are better the slower one elutes since it gives the sample more time to diffuse out from the core of the particles. While I give minimum volume (range) recommendations, the safest recovery would be to use a larger volume and concentrate.

- Finally, there is the issue of bulk binding vs. plugged flow chromatography. For columns without a top frit or for columns with a frit which can migrate upwards over time or from pipetting (from a tapered tube), the binding of sample to loose packing is less efficient than forcing it through a tight, defined mass of (robotically loaded) packing. Chemical process engineers have concluded that plugged flow is a more efficient process method since it controls the variables in fluid flow. BioPureSPN have parallel walls so there isn't an upwards force to displace the frits and they have an upper frit to constrain the packing. Overall, we feel the design is superior for long term storage of unused columns and for better performance from a fluid flow perspective compared to the alternative designs for micro-volume SPE.

I want you to be successful by understanding how these things work in SPE. I hope this helps reassure you that your hard work to obtain your samples will not be compromised and that I have provided the best products to protect them. Be sure to mark the outside of the tubes so that they are put into the centrifuge with the same orientation each time.

Thank you for asking these questions comparing the web site listing, operating instructions and label designations.