A) Quantification basis

1. **Is reported quantity based on absolute calibration against a reference standard, or relative/normalized signal?**

   We perform a system calibration using reference materials. We then verify the validity of the calibration with a check standard each run.

2. **Do you average replicate injections or preparations before reporting a single value?**

   No, our data is reported from a single preparation/single injection. We do offer a multiple vial/multiple injection option but our standard data is from a single vial/single injection

B) Sample preparation

3. **Do you assume full recovery of peptide upon reconstitution, or do you measure recovery implicitly?**

   There is really no way to measure recovery from the vials without using something like isotope dilution and the isotopic standards do not exist at the moment; thus, all of the labs are forced into a system where you have to assume that 100% of the peptide in the vial goes into solution. Note that the end user makes this assumption, so any method other than assuming full recovery of the peptide does not accurately reflect the ultimate use of the vial.

4. **Do you correct for adsorption, losses, or degradation, or are these reflected in the final number?**

   Again, there is really no way to do this given the samples as they are received. The assumption inherent in the production of the vials and therefore in the testing protocols is that 100% of the peptide (and impurities) are present in the solution and that there is no degradation at the time of analysis.

C) Detection & calculation

5. **Which detector(s) are used for quantification (UV, MS, both), and which signal is authoritative for quantity?**

   We use both UV and MS; we use them for different measurements. We use the UV signal for quantification of the peptide and impurities. We use the MS for spectral confirmation of the identity of the peptide.

6. **How is purity incorporated into quantity (e.g., normalization vs independent measurement)?**

   Purity and quantification are two separate concepts. We report a "chromatographic purity" value, which is simply the area of the peptide ratioed to the total area of all integratable peaks in the chromatogram. The number of assumptions present in this measurement are such that this

value is really not very useful. Note that this measurement is inherently normalized since you take the total integratable area as representing 100% of what is present in the vial, which is clearly bogus.

We measure the peptide mass by comparing the area of the peptide of interest against the area of a known standard. This measurement is not normalized; we look at absolute response and calculate the mass of the peptide from that response.

D) Precision & uncertainty

7. **What is your stated margin of error for this peptide, and does it include preparation + calibration uncertainty?**

   Our acceptance criteria for our check standard is +/-10% of the nominal value. This sets the lower limit of error in the analysis at 10%, which does include preparation and calibration uncertainty. It does not include inter-vial variability since our routine tests are single vial analyses.

8. **Are reported values rounded, truncated, or raw calculated outputs?**

   We round all values to reflect significant digits. Industry convention has been to report chromatographic purity to 4 significant figures; no one using commercially available instrumentation can get anywhere close to that. We estimate that our chromatographically-derived values are valid to two significant figures.

E) Interpretation

9. **Does your reported "mg per vial" represent recovered mass, inferred mass, or label-equivalent mass?**

   Since the assumption is that 100% of the mass is in solution it represents the mass recovered from the vial using reference materials as standards. We do also report a label claim comparison; we also report our detected mass as a percentage of the label mass.

10. **Under what conditions would you expect two vials from the same batch to differ by <1%?**

    Statistically it is possible but given the measurement uncertainty (MU) inherent in the analytical scheme plus the variability in the production of the vials it is unlikely. All analytical data is statistical in nature so obviously you will have situations where the measured mass is within 1% between two vials but on average you would expect the differences to reflect, at a minimum, your MU. Since the MU does not take into account variations in the production of the vials you actually would expect, on average, to see values higher than your analytical MU between two (or more) vials.