Protein Precipitation (A different form of sample preparation) Part 1 of 2

Proteins are solvated in aqueous via polar interactions with water molecules. Proteinaceous samples such as plasma are frequently analyzed for small molecule drugs and the corresponding metabolites via a technique known as protein precipitation (PPT). PPT can be for cleanup of many different types of matrices, like in Food/Bev, BioAnalytical, etc.

PPT can be performed with an organic solvent or strong acids or salts by forcing proteins to denature and associate with other proteins rather than remain in solution. Protein balling and falling out of solution, precipitation, is useful for selectively removing protein from a sample or for reducing the interaction or non-specific binding of a small molecule analyte with a binding protein in plasma, like with vitamin D and many other small molecules in plasma with propensity to associate with plasma proteins.

Different species of plasma (dog, rat, human, monkey) are frequently analyzed by pharmaceutical companies via PPT alone or PPT+SPE prior to their analytical technique such as LC/MS/MS.

PPT is less selective than SPE for cleanup and requires sample dilution but it is also simpler, less expensive and quicker than SPE.

PPT Method Summary

**Organic Based Precip Agent** - (This is the best option for dog, rat, human, monkey plasma)

- Ideal for Ion Exchange SPE (X-C, X-AW, SCX, etc.) no dilution needed usually
- Okay for Neutral SPE (C18-E, X, C8, etc.) dilution is needed though to reduce organic strength prior to loading on SPE

Best = Acetonitrile (analytes must be stable in organic) avoid methanol as precip is incomplete

1. Add ≥2:1 Ratio of Precip Agent to Plasma Sample
2. Agitate for protein to fall out of solution and then centrifuge to a pellet and remove supernatant for analysis or use high-throughput filter plate like Strata Impact.

**Aqueous Based Precip Agent** - (Best options for dog, rat, human, monkey plasma)

- Ideal for Neutral SPE (C18-E, X, C8, etc.)
- Okay for Ion Exchange SPE (X-C, X-AW, SCX, etc.) pH or salt adjustment needed prior to loading on SPE

Best = 20% TCA in water (analytes must be stable in acid)

1. Add ≥2:1 Ratio of Precip Agent to Plasma Sample
2. Agitate for protein to fall out of solution and then centrifuge to a pellet and remove supernatant for analysis or use high-throughput filter plate like Strata Impact.

Best = Zinc Sulfate (analytes must be stable in zinc sulfate)

1. Add ≥2:1 Ratio of Precip Agent to Plasma Sample
2. Agitate for protein to fall out of solution and then centrifuge to a pellet and remove supernatant for analysis or use high-throughput filter plate like Strata Impact.

For any questions regarding PPT please email the SamplePrepSupport@phenomenex.com

Also, refer to:
Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography–tandem mass spectrometry
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