



Optimization of a Protocol for Human Serum Sample Preparation for Untargeted Metabolomics Profiling Using Bradford Assay

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Abstract

Protein precipitation is the most common sample preparation method applied in studies involving biological matrices, such as plasma and serum, in metabolomics. However, a poor protein precipitation step may reduce the lifetime of chromatographic columns, may cause interferences in Mass Spectrometry, and may result in limited access to sample metabolites. In this work different protein precipitation approaches were evaluated in order to assess the best protocol for untargeted metabolomics studies. For this purpose, solvents selection; sample volumes; sample/solvent ratio and temperature were evaluated by a factorial planning. Serum samples were analyzed by Bradford assay.

Key words:

Bradford assay, Sample preparation, Metabolomics.

Introduction

Metabolomics is the study of the metabolites present in biological samples aiming for the evaluation of systematic metabolic responses to environmental changes, toxic stimuli, genetic alterations, or presence of diseases. Sample preparation is a very important step in untargeted metabolomics. Protein precipitation is the most applied sample preparation step and influences the obtained data. Different solvents may be used for protein precipitation. Temperature, solvent volume, and solvent/sample ratio can also affect the process of protein precipitation. Despite the importance of these variables, there are only a few studies in literature on this subject. The Bradford assay for protein quantification is a well established method that estimates protein concentration quickly and accurately by using a spectrophotometer and Coomassie brilliant blue reagent.

Results and Discussion

A pool of serum samples from healthy volunteers was obtained at Campinas' Blood Center. Four variables were investigated concomitantly by a factorial planning, namely: organic solvents (methanol, acetonitrile, and acetone); solvent:sample volume ratios (1:1 and 1:2); sample volumes (80 and 100 μ L); temperature (room and ice cold). Combination of these variables resulted in 72 different assays. Serum sample proteins were precipitated with organic solvent, the supernatant was transferred to a microcentrifuge tube and was dried using a speed vacuum. Before analysis of real samples, an analytical curve was prepared using BSA (Image 1). Serum samples were analyzed by UV-visible spectrophotometry, to obtain the absorbance values, and calculate protein concentration from the analytical curve. Proteins concentration in all samples were extremely low, which shows the efficiency of the sample preparation step. Sample number 16, which contained 80 μ L of serum treated with 80 μ L of ice cold methanol, in a sample:solvent ratio of 1:1, presented the highest concentration of remaining protein. Therefore, sample number 16 indicates the least recommended conditions for

serum protein precipitation, according to the preliminary data.

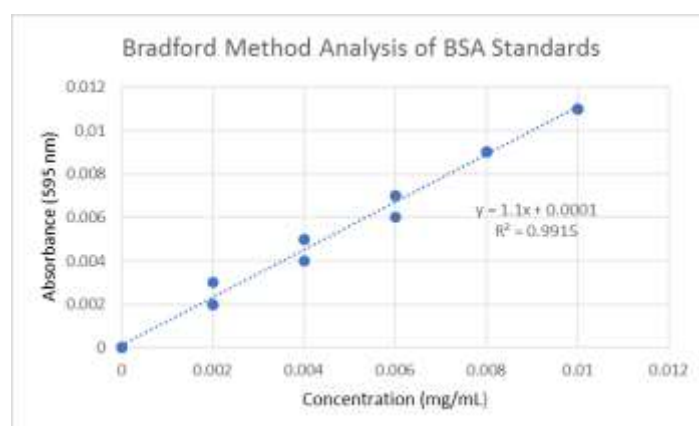


Image 1. Analytical Curve of BSA Standard Samples.

Conclusions

The results obtained by far indicate that the least recommended condition for protein precipitation is 80 μ L of serum treated with 80 μ L of ice cold methanol, in a sample:solvent ratio of 1:1. These results will be used for the next steps of the optimization protocol for serum sample preparation. A split plot approach is underway to evaluate the most appropriate solvent (according to the triangle solvents) and, to each point, a factorial planning (2^3) will be performed. Therefore, 4 factors will be varied concomitantly.

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