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The Effect of Ion Concentration on the Linearity of 25% Sulfosalicylic Acid Standard Curve

Jinadasa A.G.R.G.[1], Siriwardhana I.D.[2#], Gunawardana K.B.[1], Attanayake A.P.[3]

[1]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna*
[2]*Department of Pathology, Faculty of Medicine, University of Ruhuna*
[3]*Deparment of Biochemistry, Faculty of Medicine, University of Ruhuna*

[#]***Corresponding author:*** *deepani@med.ruh.ac.lk*

Background: Sulfosalicylic acid (SSA) test is a well-established semi-quantitative method used for the determination of the total protein content of urine. Previously 25% SSA method was optimized for quantitative determination of total protein of urine in the lower range using a manual spectrophotometric analysis based on a linear standard curve, having distilled water diluent in a dependent dilution series. However, the effect of ion concentration of the diluent on the linearity of the standard curve was not assessed. İt is important to find this relationship, since the analytical sensitivity of the method is dependent on the linearity of the curve.

Objectives: To evaluate the effect of ion concentration of the diluent on the linearity of the standard curve in modified 25% SSA method.

Methodology: A 200 mg/dL stock solution of bovine serum albumin factor V was prepared in 0.9% sodium chloride and 0.1% sodium azide solvent. The following diluents were used to prepare dependent and independent dilution series of albumin using the above stock solution. I) 0.9% sodium chloride in 0.1% sodium azide. II) 0.45 % sodium chloride in 0.1% sodium azide III) Distilled water. The protein concentrations were measured using modified 25% SSA test. The standard curves were generated for each diluent using Minitab16 statistical software. The best curve was selected considering the linearity range and R^2 value of each standard curve. The Intra and inter assay precisions of the selected curve were assessed using two QC levels for method validation.

Results: It was observed that the degree of non-linearity of the standard curves increased with increasing ion concentration of the diluent. Highest linearity was observed with distilled water diluent which yielded a linerity range of 0-50 mg/dL, where R^2= 100% for dependent dilution series.

Conclusions: The linearity of the standard curve is affected by the ion concentration and the best linearity was observed with distilled water diluent.

Keywords: Linearity, Sulfosalycilic acid, Ion concentration