



Research article

A multi-component spectrophotometric method for simultaneous determination of conjugated bilirubin, unconjugated bilirubin, oxyhemoglobin and methemalbumin in adult human serum

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Key words: Total bilirubin, unconjugated bilirubin, conjugated bilirubin, oxyhemoglobin (HbO₂), methemalbumin (Mha), serum, adults.

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Abstract

A new multi-component spectrophotometric method was developed to determine the accurate concentrations of the conjugated bilirubin (CB), unconjugated bilirubin (UB), oxyhemoglobin (HbO₂) and methemalbumin (Mha) in adult human serum of a healthy subject. The method of preparation of serum solutions has been developed, like the use of distilled water as a solvent and centrifugation of serum solutions to clear the sample turbidity. The results of bilirubins were compared to the diazo assay, as a reference method. The formulas used for the calculation of the major components (UB, CB, HbO₂ and Mha) in human serum have been derived based on the theory of multi-component spectrophotometric analysis and the mathematical Gaussian elimination method for matrix calculation. The method of multi-component spectrophotometry (at $\lambda=450, 490, 576, 600$ and 700 nm), suggested in this study for determination of total bilirubin (TB), UB and CB showed small % error of values 0.201, 2.772 and 7.81%, respectively, indicating the high accuracy of the method. The method showed small coefficients of variation (CV=3.84-10.5%), indicating the high precision of the method. The method is inexpensive, precise, accurate, and reproducible and has the advantages of small sample volume, simplicity speed and can be computerized.