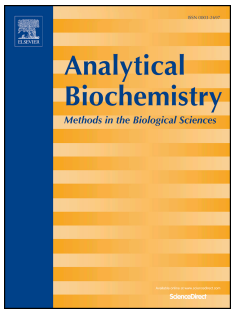
Accepted Manuscript



Development, validation and application of first derivative spectroscopy ratio method for estimation of Bradford assay

Pece Sherovski, Goran Stojković, Natasha Ristovska

PII: S0003-2697(18)30199-4

DOI: 10.1016/j.ab.2018.07.027

Reference: YABIO 13095

To appear in: *Analytical Biochemistry*

Received Date: 24 February 2018

Revised Date: 1 July 2018

Accepted Date: 31 July 2018

Please cite this article as: P. Sherovski, G. Stojković, N. Ristovska, Development, validation and application of first derivative spectroscopy ratio method for estimation of Bradford assay, *Analytical Biochemistry* (2018), doi: 10.1016/j.ab.2018.07.027.

DEVELOPMENT, VALIDATION AND APPLICATION OF FIRST DERIVATIVE SPECTROSCOPY RATIO METHOD FOR ESTIMATION OF BRADFORD ASSAY

Pece Sherovski[1], Goran Stojković[1], Natasha Ristovska[1]

e-mail: sherovski.p@hotmail.com
[1]Institute of Chemistry, Faculty of Natural Sciences and Mathematics,
Ss. Cyril and Methodius University, Skopje, Macedonia

Abstract

Several, novel and different spectrophotometric methods based on UV and first-order derivative spectrophotometry, were developed to increase accuracy and improve sensitivity of the Bradford assay for protein quantification in hair samples. The linear calibration function was established in concentration range 2-90 μg/mL at pH 0.6.

Significant improvement over the classical method was observed using three different methods based on first-order derivative spectra: signals at 550 nm ($^1D_{550}$), numerically integrated area under curve from 545 to 565 nm ($AUC^1D_{545\text{-}565}$) and the ratio of peaks at 550 and 420 nm ($^1D_{550}/^1D_{420}$). The correlation coefficients were 0.9924, 0.9932 and 0.9991, respectively. Accordingly, the best correlation was observed by the $^1D_{550}/^1D_{420}$ ratio method. The LOD of 15.67 μg/mL ($^1D_{550}$), 14.80 μg/mL ($AUC^1D_{545\text{-}565}$), 5.45 μg/mL ($^1D_{550}/^1D_{420}$) and LOQ of 47.48 μg/mL ($^1D_{550}$), 44.85 μg/mL ($AUC^1D_{545\text{-}565}$), 16.51 μg/mL ($^1D_{550}/^1D_{420}$), were calculated.

The sample recoveries for $^1D_{550}/^1D_{420}$ ratio method have proven that its accuracy (95.05%) is within the acceptable interval of recoveries. The obtained 1.55% RSD for precision fell well within the criteria accepted in bioanalytical method validation. This method was applied for determination of total proteins in hair samples. Also, the influence of different chemical treatments on total protein amount in hair sample was investigated.

Keywords: Derivative spectroscopy ratio method, Bradford assay, hair proteins

1. Introduction

Depending on its moisture content (up to 32% by weight), the human hair, consists of approximately 65% to 95% proteins characterized with many disulfide bonds [1], [2]. These proteins are classified as fibrous structural keratin proteins of acidic Type I and neutral-basic Type II, with a molecular weight of 40-65 kDa, and keratin-associated proteins (KAP's) which include high-sulfur, ultra-high sulfur and high-glycine-tyrosine, with a molecular weight of 6-30 kDa [3], [4]. The remaining constituents of hair fiber are water, structural and free lipids, pigment and trace elements, that are generally combined chemically with side chains of protein groups or fatty-acid groups [5].

Furthermore, excessive use of chemicals for permanent waves, chemical bleaches, alkaline straighteners and sunlight exposure can be influenced on the hair quality and its damage. Chemical hair treatment caused the disconnecting of naturally existing –SS– groups and many of the proteins are fragmented [6], [7], [8]. Many researchers have attempted to improve hair protein chemistry by developing convenient procedures for isolation, separation and quantification of keratins and KAP's [9].

Colorimetric methods are frequently used for quantification of protein solutions as they are rapid and inexpensive, do not require a hydrolysis step, and frequently show good correlation to more high-priced and time-consuming techniques HPLC/MS/MS [10], [11], MALDI-TOF [12], 2DE/MS [13]. On the other hand, the presence of non-protein buffers and reagents, detergents, salts and reducing agents may cause band distortion and poor protein resolution. Therefore, the decisions on the usefulness of any spectrophotometric procedure