Arabian Journal of Chemistry (2013) xxx, xxx–xxx

King Saud University

Arabian Journal of Chemistry

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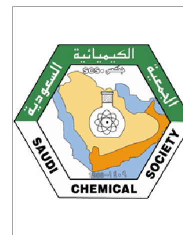

ORIGINAL ARTICLE

Spectrophotometric assay of creatinine in human serum sample

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Received 31 October 2012; accepted 20 July 2013

KEYWORDS

Creatinine;
Metol;
Serum;
Jaffe's

Abstract A new spectrophotometric method for the analysis of creatinine concentration in human serum samples is developed. The method explores the oxidation of p-methylamino phenol sulfate (Metol) in the presence of copper sulfate and creatinine which yields an intense violet colored species with maximum absorbance at 530 nm. The calibration graph of creatinine by fixed time assay ranged from 4.4 to 620 μM. Recovery of creatinine in human serum samples varied from 101% to 106%. Limit of detection and limit of quantification were 0.145 μM and 0.487 μM respectively. Sandell's sensitivity was 0.112 μg cm^{-2} and molar absorptivity was 0.101×10^4 L mol^{-1} cm^{-1}. Within day precision was 2.5–4.8% and day-to-day precision range was 3.2–7.8%. The robustness and ruggedness of the method expressed in RSD values ranged from 0.78% to 2.12% and 1.32% to 3.46% respectively, suggesting that the developed method was rugged. This method provides good sensitivity and is comparable to standard Jaffe's method with comparatively less interference from foreign substances.

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1. Introduction

Creatinine a break-down product of creatine phosphate is a nontoxic biomolecule with no significance in biological metabolism. Filtration of creatinine in blood is done by the kidney without any reabsorption. Any significant decrease of the efficiency of filtration by the kidney is identified by an increase in the concentration of creatinine in the blood. Thus, creatinine levels in the blood and urine are used to calculate the creatinine clearance which reflects the glomerular filtration rate (GFR). Estimation of GFR is the most widely used test for renal function in clinical practice (Zamora et al., 2007).

Abbreviations: Metol, p-methylamino phenol sulfate; QCM, quality control material

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.arabjc.2013.07.030

Jaffe's reaction involving the alkaline sodium picrate method is the widely accepted for creatinine measurement (Parvesh et al., 1981). This method lacks in sensitivity, reproducibility, and precision due to endogenous and exogenous interferants (Weber and Zanten, 1991). Several modifications were effected to Jaffe's method to eliminate the drawbacks. These included the specific adsorption of creatinine, removal of interfering compounds, dialysis, varying the pH, and kinetic measurements. But, none of these modifications could successfully eliminate the interferants present in varying concentrations in biological samples.

Jaffe's method has been modified using multienzyme systems to improve the specificity of creatinine determination. Under optimum conditions this enzymatic method gives accurate results but they are less precise and also of high cost. The use of multi-enzyme systems requires caution, as the risk of interference by enzymes increases with the use of more number of enzymes (Thompson et al., 1974; Moss et al., 1975).

There are other methods for the assay of creatinine which include the use of reagents such as 3,5-dinitrobenzoic acid (Langley and Evans, 1936; Benedict and Behre, 1936), 3,5-dinitrobenzoyl chloride (Parekh et al., 1976; Critique et al., 1977), methyl-3,5-dinitrobenzoate in a mixture of dimethyl sulfoxide, methanol, and tetramethyl ammonium hydroxide (Sims and Parekh, 1977), 1,4-naphthoquinone-2-sulfonate (Sullivan and Irreverre, 1958; Cooper and Bigga, 1961; Conn, 1960), Sakaguchi's color reaction of creatinine with o-nitrobenzaldehyde (Pilsum et al., 1956; Pilsum, 1959) and mass fragmentography (Bjorkhem et al., 1977).

We here propose a new spectrophotometric assay method for the estimation of creatinine. The main objective in developing this method was to obtain better sensitivity, more convenience and simultaneously overcoming the need for costly equipment and reducing the time gap. The reagent metol used in this study is readily water soluble, economic and is less toxic when compared to other aromatic reagents reported for the creatinine assay. Metol can be selectively oxidized to yield a chromogenic product (Allinson, 1943). Using metol in the assay simplifies the estimation of creatinine in the human serum sample, as it is a single reagent method. During this experimental study we performed linearity, precision, accuracy, standard deviation and interference tests, applicability of the method is carried out in serum samples and the related data are compared with the routine pathological kit method.

2. Materials and methods

2.1. Apparatus and reagent preparation

A JASCO model UVIDEC – 610 PC spectrophotometer with 1 cm matched quartz cells was used for all measurements. pH of the solution was maintained using chemlabs (Nairobi, Kenya) pH meter. All chemicals used in the assay were of analytical grade. Reagents were freshly prepared using double distilled water and stored in amber colored standard flasks and refrigerated at −4 °C until use. Metol solution (Himedia. Ltd, India) (58 mM) was prepared by dissolving 200 mg in 10 ml distilled water. Copper solution (Himedia) (2.06 mM) as copper (II) sulfate pentahydrate was prepared by dissolving 5 mg in 10 ml of distilled water.

Creatinine was purchased from S.D Fine Laboratory, Mumbai, India and the required concentration was prepared by dissolving in double distilled water. Quality control material (QCM), Lot No. 179800, used as calibrator in Roche/Hitachi Systems used in modified Jaffe's method was purchased from Cobas, UK, and diluted to the required concentration at the time of experimental study. A stock solution of 10 mM acetate buffer of pH 5.4 was prepared and used throughout the study.

2.2. Analytical method development

Different media were investigated to develop a suitable spectrophotometric method for the analysis of creatinine concentration. For selection of media the criteria employed were sensitivity of the method in a particular pH, ease of sample preparation, interference of excipients, cost of reagents and applicability of the method. Absorbance of reaction product in the selected medium at respective wavelength was determined, apparent molar absorptivity; Sandell's sensitivity, robustness and ruggedness were calculated according to the standard formulae

2.3. General procedure for the estimation of creatinine

Calibration graph for the estimation of creatinine was carried out in a final 3 ml reagent mixture containing 1.93 mM Metol, 68.6 μM copper and 1 mM acetic acid/sodium acetate buffer of pH 5.4. The reaction was initiated by adding 100 μL of various concentrations of creatinine. The reaction mixture was allowed to stand for 30 min at room temperature. Absorbance of the colored solution was recorded at 530 nm.

2.4. Precision studies

Precision studies were carried out to evaluate the magnitude of total precision of the proposed method. A final 3 ml solution containing optimized reagent concentration along with varying amounts of creatinine within the linearity range was employed for the precision studies. Three different concentrations of creatinine of 88, 265 and 442 μM were selected to analyze the precision of the proposed method. The study included 10 runs in a day with a time interval of 1 h for within day precision and 20 days run for day-to-day precision with a time interval of 24–25 h. All solutions were prepared freshly every day.

2.5. Sandell's sensitivity (*S*)

Sandell's sensitivity (Bode, 1991), $S = 10^{-3}/\varepsilon_s = \mu g\ cm^{-2}$

where ε_s is the specific absorptivity and its value (in $mL\ g^{-1}\ cm^{-1}$) corresponds to the determinant in a cuvette with an optical length of 1 cm.

Also, ε_s = (ε/molecular weight of creatinine) × 1000, where ε = molar absorptivity = A/Cd, where C is the molar concentration of the determinant and d = 1 cm path length.

3. Results and discussion

3.1. Absorption spectra

The reagents for measuring absorbance spectra were prepared as described in the general procedure. The absorption spectra

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