14.3 Estimation of creatine & creatinine

Estimation of creatinine

Principle

An alkaline picrate solution is added to a filtrate of urine and the creatinine contained therein reacts to form a complex orange-red coloured compound (Jaffe Reaction). The colour may be due to formation of picramic acid or to an enolized form of the picric acid itself.

Reagents

1. NaOH solution (10% w/v): Dissolve 10 g of NaOH in water and dilute to 100 ml.
2. Picric acid (saturated): Dissolve 13 g picric acid to 800 ml hot distilled water, cool and makeup the volume to 1 litre.
3. Alkaline picrate solution: Mix 5 volumes of saturated picric acid solution to one volume of NaOH.
4. Standard solution:

Stock solution : Creatinine solution is prepared by dissolving 1.0 g of pure dry creatinine in 0.1 N HCl and diluted to 1 litre with acid.

Working solutions : Dilute 3 ml of stock solution to 500 ml with water using 50 ml of 0.1 N HCl (0.006 mg/ml).

Procedure

1. Dilute urine with water in a ratio of 1:20 and filter. Add 1 ml alkaline picrate solution to 2 ml filtrate, mix well and allow the colour to develop for 20 min at room temperature.
2. Include a blank tube containing 2 ml water & 1.0 ml of alkaline picrate solution.
3. Standard course is prepared in the same way by taking 0.5, 1.0, 1.5 and 2.0 ml working standard and adding water to make volume 2.0 ml.
4. Read the O.D. of unknown and standards against blank at 520 nm. The colour is stable for at least 30 min.

Calculation

$$\text{Urine creatinine (mg/100 ml)} = \frac{\text{O.D. of unknown}}{\text{O.D. of known}} \times \text{Dilution factor} \times 100$$

Estimation of creatine

Principle

The creatine in the presence of mineral acid is transformed into creatinine by autoclaving. By determining the creatinine content before and after treatment with acid, the amount of creatine present may be obtained by difference.

Reagent

1 N HCl

Procedure

- Add 2 ml diluted urine and 1.0 ml of 1 N HCl in stoppered test tube and autoclave the tube at 14 lb pressure at 120ºC for 20-30 min.
- Determine the creatine as described above.

Calculation

Urine creatine = Total creatinine – Preformed creatinine

Reference: Wootton, I.D.P. 1964. Micro- analysis in Medical Biochemistry, 4th edn., J. and A. Churchill Ltd., London, pp. 174 – 175.