QUANTITATIVE DETERMINATION OF 17-KETOSTEROIDS IN URINE

Principle:

17-ketosteroids (steroid hormone metabolites) occur in urine in the form of glucuronides and sulfates. The conjugates are cleaved by means of acidic hydrolysis and the steroids are extracted with tetrachloromethane (TCM). Contaminants and chromogens are eliminated with potassium hydroxide. After CCl_4 evaporation, the 17-ketosteroids are put through the Zimmermann reaction (a chromogenic reaction of ketones with *m*-dinitrobenzene – DNB) and determined photometrically at 520 nm.

Reagents and other solutions:

- 2% methanolic solution of *m*-dinitrobenzene (DNB)
- 5M methanolic solution of KOH
- methanol
- standard sample: androsterone (corresponding to 10 mg/l in the urine)

Procedure:

You have received three test tubes:

- evaporated sample of urine (after hydrolysis and TCM extraction)
- standard
- blank

Add 0.4 ml 2% DNB and 0.3 ml 5M KOH into each of the test tubes and mix.
Put the test tubes into the thermostated water bath (37 ˚C) for 15 minutes.
Then add 3.0 ml methanol to each test tube, mix and measure the absorbance against the blank at 520 nm.

Calculation: $$c_{\text{sample}}(\text{mg/l}) = \frac{A_{\text{sample}}}{A_{\text{standard}}} \cdot c_{\text{standard}} =$$

Complete the calculation of the 24 h excretion.

Normal values (17-ketosteroids excretion per 24 hours)**:**

- men – 12–18 mg/24 h
- women – 7–14 mg/24 h

History of the samples

The urine is collected for 24 hours, mixed, and 50 ml of it is used for the assay.

What other data do you need to evaluate the results?