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Diploma in Pharmacy 2nd Year
Biochemistry & Clinical Pathology
Experiment
To determine the SGOT/SGPT in blood/serum.

Aim:

To determine the SGOT/SGPT in blood/serum.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 59 – 64

Materials Required

0.1 M phosphate buffer having pH 7.4, distil water, monopotassium phosphate anhydrous, disodiumphosphate solution, mono potassium dihydrogen solution, aspartic acid, oxoglutaric acid, NaOH, chloroform, alanine, oxaloacetate, L partate, alpha-ketoglutarate, 2,4-dinitrophenyl hydrazine, 2,4- dinitrophenylhydrazone, sodium pyruvate, DNPH, pipette, small beaker, incubator and volumetric flask.

Theory :

The enzyme known as serum glutamic-oxaloacetic transaminase (SGOT/AST) is found in the kidneys, heart, skeletal muscles, and liver cells. When there is an injury. these enzymes are released into the tissues through the blood stream. Heart and liver cells contain serum glutamic pyruvic transaminase (SGPT/ALT), which is released into the blood when there is damage. The following two methods are used:

1) **Reitman and Frankel Method:** L-aspartate and alpha-ketoglutarate are produced when SGOT is transformed into glutamate and oxaloacetate, respectively. In an alkaline medium, this oxaloacetate

reacts with 2,4-dinitrophenyl hydrazine to produce 2,4-dinitrophenylhydrazone. It turns brown colour by producing hydrozone derivative and pyruvate standard, a calibration curve.

2) **Calorimetric Method:** DNPH is higher in oxaloacetate and pyruvate with oxoglutarate and supports the intensity of colour limiting the error.

Reagent Preparation

1) **Disodium Hydrogen Phosphate Dihydrate (0.1M):** 8.9 g of disodium hydrogen phosphate dehydrate should be dissolved in 200 ml water in 500 ml volumetric flask.
2) **Monopotassium Phosphate Anhydrous (0.1M**): 1.36 g monopotassium phosphate anhydrous should be dissolved in 50 ml of distilled water and the solution should be diluted to 100 ml. 420 ml disodiumphosphate solution should be mixed with 80 ml mono potassium dihydrogen solution.
3) **Substrate for SGOT:** 2.66 g DL aspartic acid and 30 mg oxoglutaric acid should be dissolved in 20.5 ml of 1 N NaOH in a beaker by adding drop wise to adjust pH. This solution should be then transferred into 100 ml in a volumetric flask with phosphate buffer solution. Iml of chloroform should be added as a preservative and then it should be refrigerated. The substance should be discarded if it becomes turbid.
4) **Substrate for SGPT:** 1.78 g DL alanine and 30 mg oxoglutaric acid should be dissolved in 20 ml phosphate buffer. 1.25 ml of 0.4 N NaOH should be taken in small beaker and the volume should be maintained up to 100 ml with buffer. I ml chloroform should be added as a preservative.

Procedure

1) **Pyruvate Standard**
2) **Stock Standard:** 220mg sodium pyruvate should be treated with 100ml of phosphate buffer, which should be discarded after preparing the working standard.
3) **Working Standard:** 10ml stock standard should be added to 100ml phosphate buffer and 2ml should be dispensed in a test tube and refrigerated using stopper. One tube should be used for preparing the calibration curve and the rest should be refrigerated. Remaining solution should be discarded. 200mg DNPH should be dissolved in IN HCI (diluting 90ml concentration with water). Distilled water should be added to make up the volume up to 1L..
4) **Sodium Hydroxide Solution 1N:** 40gm of sodium hydroxide should be added in 500ml of water and the volume should be maintained up to 1000ml the substrate should be pipetted out into a test tube, which is then incubated in a water bath at 40°C for 10 minutes. 0.2ml of serum should be mixed and incubated for 60 minutes (GOT) and 30 min (GPT) then the test tube should be removed. DNPH should be added to stop the reaction for 20 minutes at room temperature 110ml of 0.4N sodium hydroxide should be added and rubber stopper should be placed to mix by inverting (A).

Calculations

For SGPT:
(A_1-A_2) X 857U/L
For SGOT: (A_1-A_2) X 514 U/L

Interpretation

When the optical density changes by more than 500 mu ml against transaminase then it is noted and the normal value is SGOT, SGPT: 0-50 U/L, moderate range: 400-2000 U/L, high range 2000-30,000 U/L.

Result :

SGOT/SGPT in blood/serum was determined.