United States Patent Office 3,458,403 Patented July 29, 1969

3,458,403

METHOD AND A PREPARATION FOR THE CLINICAL DIAGNOSIS

Nobuhiko Katsunuma, Tokushima-shi, Japan, assignor to Chugai Seiyaku Kabushiki Kaisha, Tokyo, Japan, a corporation of Japan.

No Drawing. Filed May 5, 1966, Ser. No. 547,790
Claims priority, application Japan, May 11, 1965, 40/27,105, 40/27,106
Int. Cl. C12k 1/06
U.S. Cl. 195—103.5 10 Claims

ABSTRACT OF THE DISCLOSURE

This invention relates to a method for the determination of the activity of glutamic-keto acid transaminase except glutamic-oxalacetic transaminase in a body sample characterized in mixing an agent consisting of keto acid, glutamic acid, aspartic acid and glutamic-oxalacetic transaminase with a sample solution to form oxalacetic cid and recording the amount of the formed oxalacetic acid.

This invention relates to a novel method and a preparation for the clinical diagnosis, particularly relates to a method for the determination of the glutamic-keto acid transaminase activity and a preparation therefor.

The term "glutamic-keto acid transaminase" used herein defines glutamic-keto acid transaminase except glutamic-oxalacetic transaminase.

It is known that the activity of glutamic-keto acid transaminase in tissues or body fluids is varied in case of various diseases of the heart, the liver or the like, and for this reason the determination of glutamic-keto acid transaminase activity has been carried out in the diagnosis of these diseases.

Hitherto, two types of method have been routinely used for the determination of glutamic-pyruvic transaminase activity in clinical and biochemical investigations. These methods are based on different principles, being (a) a method using spectrophotometry: (b) a method involving the color developed by 2,4-dinitrophenylhydrazone of either unreacted alpha-keto acids or those formed. The former is an accurate and simple method, but it requires an ultraviolet spectrophotometer for the estimation of optical density of 340 mμ. Accordingly, when a large number of assays must be done, such as in clinical investigations, it takes much time. The latter method has the advantage that it does not require an ultraviolet spectrophotometer, but it is less sensitive and inaccurate.

Accordingly, an object of this invention is to provide a novel method for the determination of glutamic-keto acid transaminase activity.

A further object of this invention is to provide a simple and accurate method for the determination of glutamic-keto acid transaminase activity.

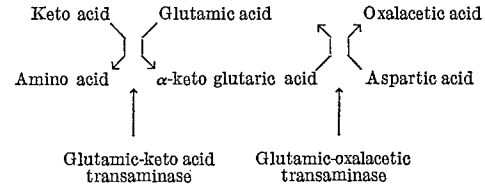
Another object of this invention is to provide a preparation therefor.

Still another object of this invention is to provide a stable and convenient preparation therefor.

The determination method of this invention comprises coupling two enzymic reactions, that is, glutamic-keto acid transamination reaction and glutamic-oxalacetic transamination reaction, converting α-keto-glutaric acid formed as the result of glutamic-keto acid transamination reaction and the added aspartic acid into glutamic acid and oxalacetic acid respectively by the glutamic-oxalacetic transamination reaction and measuring the amount of the formed oxalacetic acid.

According to this invention, the glutamic-keto acid transaminase activity may be determined by adding a keto acid or its salt, glutamic acid or its salt, aspartic acid or its salt and glutamic-oxalacetic transaminase to a suitable amount of a sample solution to cause the above mentioned enzymic reactions and measuring the amount of the formed oxalacetic acid.

The mechanism of the enzymic reactions is shown as folows:



As shown in the above mechanism, a keto acid added to the sample solution is converted into a corresponding amino acid by the action of glutamic-keto acid transaminase to be determined, accompanying the formation of α-keto-glutaric acid from glutamic acid. The formed α-keto-glutaric acid reacts with aspartic acid by glutamic-oxalacetic transaminase to form the equivalent amounts of oxalacetic acid and glutamic acid to that of α-keto-glutaric acid. Therefore, the glutamic-keto acid transaminase activity may be determined by measuring the amount of the formed oxalacetic acid.

In practice, the glutamic-keto acid transaminase activity may be determined by adding a keto acid or its salt, glutamic acid or its salt, aspartic acid or its salt and glutamic-oxalacetic transaminase to a suitable amount of a sample solution containing glutamic-keto acid transaminase, adjusting the pH in the reaction medium between about 7 and about 9, preferably 8.0 by the addition of a buffer composition, incubating at 30–40° C. preferably at 37° C. for the proper time interval, generally 6–10 minutes, to form oxalacetic acid, and measuring the amount of the formed oxalacetic acid. All of these agents may be added one by one or as a mixture or mixtures, which are previously and suitably mixed.

Since the above mentioned two enzymic reactions are reversible, it is required to take care of the amount of the substrates to be added. A keto acid or its salt, glutamic acid or its salt and aspartic acid or its salt may generally be used at the molar ratio of 0.8–3:1–5:10–30, preferably 1:2:15. Glutamic acid aspartic acids may be used either as L-form or as D,L-form. A keto acid, glutamic acid and aspartic acid may be used in the form of a water soluble salt, for example, mono- or di-lithium, sodium or potassium salts.

The amount of glutamic-oxalacetic transaminase is not critical, with the exception that a substantial excess thereof should be added. For example, when the activity in the body fluid is determined, glutamic-oxalacetic transaminase is preferably added at the concentration of 10–100M I.U. to 0.2 ml. of body fluid.

Pyridoxal phosphate may be preferably added to promote the transamination reaction.

To promote the enzymic reaction smoothly, it is preferable to use a buffer composition to keep the pH of a reaction medium during the measurement between about 7 and about 9. Tris-HCl buffer, phosphate buffer and the like may be advantageously used.

The amount of the formed oxalacetic acid may be estimated after reaction period by ordinary analytic methods, wherein the most advantageous method is a colorimetrical method using a color developer agent, especially an azonium salt.

Some of azonium salts do not inhibit the enzymic reactions, but they react with oxalacetic acid specifically under controlled conditions to form a colored coupling material which has an absorption in visible spectrum. An

azonium salt, therefore, may be added to the enzymic reaction medium before or after incubation, and the amount of the formed oxalacetic acid may be advantageously measured by using a colorimeter or visually by comparing the developed color with a standard color chart. As such an azonium salt, it may be mentioned, for example, 4-amino-2,5-diethoxybenzanilide diazonium chloride, 6-benzamide-4-methoxy-m-toluidine diazonium chloride, tetrazotized O-di-anisidine and the like. Some of azonium salts may be more advantageously used in the form of a double salt with an inorganic halide, sulfate or the like of a metal such as cadmium, manganese, zinc, magnesium and the like, for example manganese chloride, magnesium chloride, cadmium chloride, zinc chloride and the like, for the reason described below.

The amount of the formed oxalacetic acid may also be measured by using 2,4-dinitrophenylhydrazine. In this method, the amount of the formed oxalacetic acid may be determined as 2,4-dinitrophenylhydrazone derivative. It may be advantageously determined in the solution containing oxalacetic acid only, which may be prepared for example by column- or paper-chromatography.

The amount of the formed oxalacetic acid may be also measured by using a mixture of aniline and citric acid. According to this method, the formed oxalacetic acid is decomposed by the action of a mixture of aniline and citric acid into pyruvic acid and carbon dioxide and the enzymic activity may be determined by the measurement of the amount of carbon dioxide gas using Warburg's manometer.

It may be also measured by the combined above two methods. That is, the enzymic activity may be measured by decomposing the formed oxalacetic acid into pyruvic acid and carbon dioxide, and measuring the amount of the formed pyruvic acid by using 2,4-dinitrophenylhydrazine. But, when glutamic-pyruvic transaminase activity is determined, the formed oxalacetic acid must be separated from the reaction mixture to determine the amount thereof, because an excess of pyruvic acid has been added in the reaction medium.

In the measurement of the amount of the formed oxalacetic acid using an azonium salt, it is advantageous to be measured after stopping the enzymic and the color developing reactions. The enzymic reaction may be stopped by the addition of an organic solvent miscible with water, for example, methanol, ethanol, isopropanol and the like, and the color developing reaction may be stopped by controlling the pH in the reaction medium, generally by the addition of an organic and/or an inorganic acid such as acetic, trichloroacetic, sulfuric, hydrochloric acids and the like.

In general, the measurement carried out after the stopping of the color developing reaction, followed after the stopping of the enzymic reaction. But in case of using 6-benzamide-4-methoxy-m-toluidine diazonium chloride or the double salt thereof, the above two reactions may be simultaneously stopped because the optimum pH of the enzymic acid the color developing reactions are almost the same and the formed oxalacetic acid rapidly reacts with this azonium salt. In this case, accordingly, a mixture of alcohol and sulfuric acid or hydrochloric acid at the ratio of 95–99:5–1 by volume may be advantageously used as a reaction stopper agent. The enzymic reaction is inhibited by 2,4-dinitrophenylhydrazine, so that it is not required to use an enzymic reaction stopper agent to stop the color developing reaction, in case of using 2,4-dinitrophenylhydrazine. In the measurement using Warburg's manometer the use of an enzymic reaction stopper agent is not required for the same reason.

As glutamic-keto acid transaminase to be determined according to this invention, it may be mentioned glutamic-pyruvic transaminase, glutamic-p-hydroxyphenylpyruvic transaminase, glutamic-imidazolepyruvic transaminase, glutamic-glyoxylic transaminase and the like. Keto acid used in this invention must be selected according to the transaminase to be determined.

Describing a keto acid corresponding to the transaminase to be determined, pyuvic acid to glutamic-pyruvic p-hydroxyphenylpyruvic transaminase, imidazolepyruvic acid to glutamic-imidazolepyruvic transaminase, glyoxylic acid to glutamic-glyoxylic transaminase are used.

According to this invention, the enzymic activity of glutamic-keto acid transaminase may be determined simply and accurately without using an ultraviolet spectrophotometer and it is convenient when a large number of assays must be done. Especially, in case of using an azonium salt, it is very convenient in the point that it is possible to measure the formed oxalacetic acid using a colorimeter or visually comparing the developed color with a standard color chart.

Reaction agents used in this invention are as follows:

(1) Keto acid or its salt
(2) Glutamic acid or its salt
(3) Aspartic acid or its salt
(4) Glutamic-oxalacetic transaminase
(5) Oxalacetic acid determining agent
(6) Buffer composition adjusted to maintain the pH in the reaction medium during the measurement.

Other than these agents described above, pyridoxal phosphate, a reaction stopper solution (agent) and an inactive diluent may be used, if necessary.

Pyridoxal phosphate is advantageously used to promote the enzymic reaction smoothly, because it is a coenzyme of various transaminases.

These agents, (1)–(6) and pyridoxal phosphate may be added one by one, or as a mixture of mixture thereof. Generally, it is convenient to use the preparation, which is prepared by mixing some or all of these agents just before use or previously. Especially, to simplify the determination procedure, it is most advantageous to use the previously prepared preparation such as tablet, powder, granulte, liquid or the like.

As a typical suitable preparation it may be mentioned as follows.

Type A.—A set comprising as follows:

(1) A preparation comprising keto acid or its salt; glutamic acid or its salt; aspartic acid or its salt; glutamic-oxalacetic transaminase; pyridoxal phosphate; oxalacetic acid determining agent; a buffer composition adjusted to maintain the reaction medium between about 7 and about 9.

Suitable preparation form: tablet, powder and granule.

(2) An enzymic reaction stopper agent.

(3) Aspeartic acid or its salt

Type B.—A set comprising as follows:

(1) A preparation comprising keto acid or its salt; glutamic acid or its salt; asparatic acid or its salt; glutamic-oxalacetic transaminase; pyridoxal phosphate; a buffer composition adjusted to maintain the pH in the reaction medium between about 7 and about 9.

Suitable preparation form: tablet, powder and granule.

(2) An oxalacetic acid determining agent.

Suitable preparation form: tablet, powder and granule.

(3) An enzymic reaction stopper agent.

(4) A color developing stopper agent.

Type C.—A set comprising as follows:

(1) A preparation comprising keto acid or its salt; glutamic acid or its salt; aspartic acid or its salt; a buffer composition adjusted to maintain the pH in the reaction medium between about 7 and about 9.

Suitable preparation form: aqueous solution, tablet, powder and granule.

(2) A preparation comprising glutamic-oxalacetic transaminase; pyridoxal phosphate.

Suitable preparation form: tablet, powder, granule and an implement comprising porous sheet material impregnated with the above mixture.

(3) An oxalacetic acid determining agent.

Suitable preparation form: tablet, powder and granule.

(4) An enzymic reaction stopper agent.

(5) A color developing stopper agent.

Type D.—(1) A mixture comprising keto acid or its salt; glutamic acid or its salt; aspartic acid or its salt; glutamic-oxalacetic transaminase; pyridoxal phosphate; a buffer composition adjusted to maintain the pH in a reaction medium between about 7 and about 9.

Suitable preparation form: tablet, powder, and granule.

(2) An oxalacetic acid determining agent.

Suitable preparation form: tablet, powder and granule. The preparation of type A may be prepared by adding an inactive diluent, e.g. lactose, polyvinyl pyrrolidone, carboxymethylcellulose and the like, to a mixture composed of a keto acid or its salt, glutamic acid or its salt, aspartic acid or its salt, pyridoxal phosphate, glutamic oxalacetic transaminase, a buffer composition adjusted to maintain the pH in the reaction medium during the measurement between about 7 and about 9 and an oxalacetic acid determining agent to give a powder, granule or tablet form. The liquid form may be also prepared, but it is not suitable for the long preservation. In the preparation of type A. It is necessary that the oxalacetic acid determining agent does not inhibit the enzymic reaction. In this sense, the use of an azonium salt is the most suitable. And when an azonium salt, which forms a colored coupling material by reacting oxalacetic acid in the same pH range as that in the enzymic reactions is used, the second and the third stopper solutions may be mixed to make a simple reaction stopper solution as far as they are miscible, without any reaction, for example ethanol-furfuric acid mixture.

The preparation of this type is suitable for the quantitative and semi-quantitative analyses.

The method for the determination of the gutamic-keto acid transaminase activity using the preparation of type A is as follows:

At first, the composition preparation is dissolved in a small volume of distilled water. To a suitable amount of a sample solution is added the said solution and the mixture is incubated at 30–40° C., preferably at 37° C., for a proper time interval. The color develops gradually in proportion as the quantity of the formed oxalacetic acid. After the incubation, the enzymic reactions stopper solution and the color developing stopper solution are added thereto to stop the enzymic reactions and the color formation. The transaminase activity may be obtained by measuring optical density of the supernatant using a colorimeter or by comparing visually the developed color with a standard color chart.

As some of the color developer agent is relatively unstable, it is often preferable to separate to single two or three preparations. In such a case, the use of the preparation of type B or type C is suitable.

The first composition of type B is prepared by the same manner as in that of type A.

As the second oxalacetic acid determining agent, an azonium salt may be advantageously used.

The determination of a glutamic-keto acid transaminase activity may be carried out by the same manner as in the preparation of type A. The second composition in case of using an azonium salt, may be added before or after the enzymic reactions.

The preparation of type C is the most suitable one for quantitative analysis.

The first preparation of type C may be used as an aqueous liquid form or solid form such as tablet, granule or powder. An aqueous liquid form may be prepared by dissolving these substrates into a suitable amount of water, adding a buffer composition adjusted to maintain the pH in a reaction medium during the measurement between about 7 and about 9 thereinto and adding, if necessary, an inactive stabilizer to the resulting mixture.

A solid form may be prepared by mixing a buffer composition adjusted to maintain the pH in a sample solution during the measurement between about 7 and about 9 with a mixture of glutamic acid or its salt, a keto acid or its salt and aspartic acid or its salt. If necessary, an inactive diluent such as lactone, polyvinylpyrrolidone, carboxymethylcellulose and the like may be added.

Glutamic-oxalacetic transaminase in the second preparation may be used in the form of powder of pure crystalline, or a mixture of glutamic-oxalacetic transaminase and ovo-albumin. Glutamic-oxalacetic transaminase and pyridoxal phosphate may be used as an implement comprising porous sheet material impregnates with them.

The third oxalacetic acid determining agent is the same as used in the preparation of type B.

The first preparation of type D may be prepared by the same manner as in that of the first preparation in type A.

As an oxalacetic acid determining agent in the preparation of type D, 2,4-dinitrophenyl hydrazine may be advantageously used.

The method for the determination of glutamic-keto acid transaminase activity using the preparation of type D wherein the oxalacetic acid determining agent is 2,4-dinitrophenylhydrazine is as follows:

The first preparation is dissolved in a small volume of distilled water. To a suitable amount of a sample solution is added the said solution and the mixture is incubated at 30° C.–40° C. preferably at 37° C. for a proper time interval.

After the incubation, the second preparation containing 2,4-dinitrophenylhydrazine is added and the amount of the formed oxalacetic acid is measured. The color is formed by the addition of alkaline substance such as sodium hydroxide, potassium hydroxide, lithium hydroxide and the like.

After the completion of the color formation, the amount of the formed oxalacetic acid may be determined by the measurement of the optical density at 505 mμ. In this case, the enzymic reaction is inhibited by the addition thereof, so that no enzymic reaction stopper solution is required.

Other than these four types, various type may be considered on the preparation type.

Generally speaking, when the preparation is prepared just before use, any preparation form may be advantageously used. But it is generally convenient to use a suitable preparation which is previously prepared.

The preparation containing substrates only such as a keto acid, aspartic acid and glutamic acid or the salts thereof, as active ingredients may be used as an aqueous liquid form or a solid form such as tablet, granule or powder, each of which may be prepared by the same manner as in the first substrate preparation of type C.

The preparation containing the enzyme and/or an azonium salt is advantageously formed into a solid form, because the enzymic solution and the azonium salt solution are unstable in the state of aqueous solution, that is, the former is liable to be inactivated by various factors and the latter is liable to be decomposed by light or heat.

As a color developer agent, an azonimum salt, 2,4-dinitrophenylhydrazine or the like, especially an azonium salt, may be used. In case of using 2,4-dinitrophenylhydrazine, 2,4-dinitrophenylhydrazine must be separated as a single preparation, because this agent inhibits the enzymic reaction.

As an azonium salt, those as are described above may be used. Among these azonium salts, 6-benzamide-4-methoxy-m-toluidine diazonium chloride, preferably in the form of a double salt with an inorganic metallic salt, is most advantageous.

Comparing 6 - benzamido - 4 - methoxy-m-toluidine diazonium chloride with a double salt thereof, 6-benzamide-4-methoxy-m-toluidine diazonium zinc chloride, the former is unstable for humidity and heat, so that it is necessary to take care of the preparation form and its preservation condition, but the latter is more stable and reacts with oxalacetic acid specifically to form a colored coupling material, even in case the ethanol solution thereof is allowed to stand for one month at cold. 6-benzamido-4-methoxy-m-toluidine diazonium chloride reacted with oxalacetic acid

specifically gives a red colored coupling material which gives a maximum absorption at 520–530 mμ in an alkaline condition and gives a yellow colored coupling material in an acidic condition, a coupling material which shows a maximum absorption at 410 mμ at pH 6.0. Any of these color developments may be used for the measurement of oxalacetic acid, but it is advantageous to employ a red color in alkaline condition.

Development of the red color is rapid in a room temperature, and the formed colored coupling material is stable for several hours in an acidic condition. But after that, the solution often given a precipitation of colored coupling product depending upon the concentration of oxalacetic acid, and in this condition the measurement happens to become impossible. To overcome this disadvantage, the addition of ethanol over 50% gives good results, that is ethanol added dissolves the precipitate. Accordingly, the azonium salt may be advantageously used in the form of an aqueous or an organic solvent solution such as ethanol solution.

Instead of using the double salt in the state of liquid, the mixture solution of diazonium salt and an inorganic metallic salt may be also used.

This color developer agent doesn't react with α-keto glutaric acid, even if the concentration thereof is 3.3 mmol. Even if the sample of which the enzymic activity is determined is turbid with the presence of other inactive protein or the like, it is possible to carry out the determination because the color is developed also in high concentration of ethanol over 50% and the sample solution become clear by precipitation of protein by the presence of ethanol.

This invention will be widely applied for such an industrial field, for example, fermentation industries and pharmaceutical industries, and for clinical diagnosis, but also in a field as the determination of a glutamic keto acid transaminase activity is required.

Example 1.—Determination of glutamic-pyruvic transaminase activity

To 0.2 ml. of a sample solution, which was prepared by homogenizing the liver of a rat, adding 20 times volume of physiological saline thereto and centrifuging to remove debris, were added 0.1 ml. of pyruvic acid solution of the concentration of 100 μmol/ml., 0.1 ml. of 1.5% KCl-solution of glutamic-oxalacetic transaminase of the concentration of 24M I.U./0.1 ml. respectively.

Separately, 7500 μmol. of L-aspartic acid, 1000 μmol. of L-glutamic acid and 2.5 mg. of pyridoxal phosphate were added to 20 ml. of water, the pH was adjusted at 8.0 with 10% KOH solution and the whole volume of the solution was made to 50 ml. by the addition of tris-HCl buffer (pH 8.0).

To the former mixture solution was added 1.0 ml. of the latter mixture solution, the resulting mixture was incubated at 37° C. for 10 minutes. After 3.0 ml. of ethanol was added thereto to stop the reaction, 0.1 ml. of the double salt of 6-benzamide-4-methoxy-m-toluidine diazonium chloride and zinc chloride solution of the concentration of 20 mg.ml. was added to the said solution and the said solution was again incubated at 37° C. for 6–10 minutes to give red color. Then 1.0 ml. of 2 N HCl solution was added into the said solution to stop the colorization. After the said solution was subjected to centrifiguration at 3,000 r.p.m. for 10 minutes, optical density of the supernatant was measured at 520 mμ in a colorimeter.

Example 2.—Determination of glutamic-glyoxylic transaminase activity

To 3.6 ml. of distilled water were dissolved water were disolved 39.53 mg. of L-aspartic acid, 5.84 mg. of L-glutamic acid, 0.1 mg. pyridoxal phosphate, 24.2 mg. of tris-(hydroxymethyl) aminomethane, 10.0 mg. of glutamic-oxalacetic transaminase (2400M I.U./g.), 1.5 mg. of glyoxylic acid,

0.4 ml. of the same sample solution as used in Example 1, and 4.0 mg. of the double salt of 6-benzamido-4-methoxy-m-toluidine diazonium chloride and magnesium chloride were added to the said solution. After the incubation at 37° C. for 8 minutes, 6.0 ml. of ethanol and 2.0 ml. of 2 N HCl solution were added to the said solution. After the said solution was subjected to centrifugation at 3,000 r.p.m. for 10 minutes, the optical density of the supernatant was measured at 520 mμ in a colorimeter.

Example 3.—The determination of glutamic-pyruvic transaminase activity

The following solutions were pipetted into the main compartment of a Warburg cup: 1000 μmol. of L-gultamic acid, 7500 μmol. of L-aspartic acid, 0.1 ml. of 1.5%-KCl solution of glutamic-oxalacetic transaminase of the concentration of 24M I.U./0.1 ml., 2.5 mg. of pyridoxal phosphate and 0.2 ml. of serum. After 10 minutes of incubation at 37° C.: 0.1 ml. of pyruvic acid solution of the concentration of 100 μmol./ml. is tipped from the first side arm and incubated at 37° C. for 30 minutes, and then 0.5 ml. of aniline citrate reagent, which was prepared by mixing equal volumes of aniline and citric acid solution (50 g. of citric acid in 50 ml. of water), was tipped from the second side arm, and the gas evolved was measured within 10 minutes.

A control containing all the reagents except the enzymic solution was run simultaneously.

Example 4.—The determination of glutamic-pyruvic transaminase activity

To 5.0 ml. of the reaction mixture containing oxalacetic acid obtained in Example 1 was added to 5.0 ml. of 10% metaphosphoric acid and the solution was shaked and filtered.

To 4.0 ml. of the filtrate was added 1.0 ml. of 2,4-dinitrophenylhydrazine solution, which was prepared by adding 0.1 g. of 2,4-dinitrophenylhydrozine in 100 ml. of 2 N HCl, and the solution was mixed and incubated at 30° C. for 30 minutes. The reaction mixture was ice-cooled and the extract was carried out 3 times using 8.0 ml. of ethylacetate solution and further carried out using 2.0 ml. of carbonate solution, which was prepared by dissolving 50 g. of sodium carbonate and 5 g. of sodium bicarbonate into water to make the whole volume 1 l.

After 0.5 ml. of 6 N HCl solution was added to the ice-cooled extracts, the extracts were extracted with 8.0 ml. of ethylacetate.

The obtained extract was dried under reduced pressure. The dried material was dissolved in acetone, and this solution was spotted on the filter-paper to carry out paper chromatography.

2,4-dinitrophenylhydrazone	Rf
α-keto glutaric acid	0.05
Pyruvic acid	0.35
Oxalacetic acid	0.12

After the filter-paper was dried, the spot of oxalacetic acid was cut off and the section was extracted with a small volume of carbonate solution.

Optical density of the extract was measured at 505 mμ in a colorimeter.

Example 5.—A preparation for the determination of glutamic-glyoxylic transaminase acitvity

(1) Tablet A:	Mg.
L-aspartic acid	39.53
L-glutamic acid	5.84
Pyridoxal phosphate	0.10
Tris(hydroxymethyl) aminomethane	24.2
Glutamic-oxalacetic transaminase (2400M I.U./g.)	10.0
Glyoxylic acid	1.50

Lactose was added thereto up to 90 mg. of a whole quantity to tablet by an ordinary method.

(2) Tablet B.—The double salt of 6-benzamido-4-methyl-m-toluidine diazonium chloride and magnesium chloride 4.0 mg.

Lactose was added thereto to 10 mg. of a whole quantity to tablet by an ordinary method.

(3) A mixture solution of 6.0 ml. of ethanol and 2.0 ml. of N HCl.

For use, tablet A was dissolved in 3.6 ml. of distilled water and thereto was added 0.4 ml. of a sample solution, and further tablet B. The mixture was reacted at 37° C. for 8 minutes and thereto was added the third mixture solution. After the centrifugation at 3000 r.p.m. for 10 minutes, the optical density of the supernatant was measured 520 mμ in a colorimeter.

Example 6.—A preparation for the determination of glutamic-pyruvic transaminase activity

(1)

	Mg.
Aspartic acid	39.53
Glutamic acid	5.84
Pyridoxal phosphate	0.10
Tris-(hydroxymethyl) aminomethane	24.20
Double salt of 6-benzamido-4-methoxy-m-toluidine diazonium chloride and manganese chloride	4.0
Pyruvic acid	2.0
Glutamic-oxalacetic transaminase (2400M I.U./g.)	10.0

Lactose was added thereto up to 100 mg. of a whole quantity to granulate by an ordinary method.

(2) A mixture solution of 6.0 ml. of ethanol and 2.0 ml. of 2 N HCl.

For use, this tablet was dissolved to 3.8 ml. of distilled water and thereto was added 0.2 ml. of a sample solution. This mixture was reacted at 37° C. for 10 minutes and thereto was added the second mixture solution. After the centrifugation at 3000 r.p.m. for 10 minutes, optical density of the supernatant was measured at 520 mμ in a colorimeter.

What I claim is:

1. A method for the determination of the activity of glutamic-keto acid transaminase except glutamic-oxalactic transaminase, in a solution of a body sample comprising mixing an agent consisting of keto acid, glutamic acid, aspartic acid and glutamic-oxalacetic transaminase with a suitable volume of said sample solution to form oxalacetic acid and recording the amount of the formed oxalacetic acid.

2. A method claimed in claim 1, in which instead of free acids a water-soluble keto acid salt, water-soluble glutamic acid salt and water-soluble aspartic acid salt are used.

3. A method claimed in claim 2, in which the salts of a keto acid, glutamic acid and aspartic acid are selected from the group consisting of lithium salts, sodium salts and potassium salts.

4. A method claimed in claim 1, in which the keto acid, glutamic acid and aspartic acid are used in molar ratio of 0.8–3:1–5:10–30.

5. A method claimed in claim 1, in which pyridoxal phosphate is further added to the sample solution.

6. A method for the determination of a glutamic-keto acid transaminase except glutamic-oxalacetic transaminase which comprises adding an agent consisting of a keto acid, glutamic acid, aspartic acid and glutamic-oxalacetic transaminase to a suitable volume of a body sample solution to form oxalacetic acid and measuring the amount of the formed oxalacetic acid using an azonium salt.

7. A method claimed in claim 6, in which azonium salt is 6-benzamido-4-methoxy-m-toluidine diazonium chloride.

8. A method claimed in claim 6, in which the azonium salt is selected from the group consisting of 4-amino-2,5-diethoxybenzanilide diazonium chloride, and tetrazotide o-dianisidine.

9. A method claimed in claim 6, in which the azonium salt is a double salt of 6-benzamido-4-methoxy-m-toluidine diazonium chloride and an inorganic metallic salt.

10. A method claimed in claim 9, in which the inorganic metallic salt is selected from the group consisting of manganese chloride, magnesium chloride, cadmium chloride and zinc chloride.

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