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[54] **TEST COMPOSITION AND DEVICE FOR ASCORBIC ACID DETERMINATION**

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[58] **Field of Search** ..... 23/253 TP; 252/408

[56] **References Cited**

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[57] **ABSTRACT**

Test composition for determining ascorbic acid in fluids and the relative concentration of ascorbic acid present therein. The composition includes a solution containing an indicator stablized with a nitrate compound. The composition may be used as a spot test or incorporated with a carrier such as a bibulous material and dried to form a dip and read test device. An organic acid may also be included in the composition to prevent urate interference and adapt the composition for testing urine specimens.

7 Claims, No Drawings

TEST COMPOSITION AND DEVICE FOR ASCORBIC ACID DETERMINATION

BACKGROUND OF THE INVENTION

As many of our foods, and particularly fruit juices, are now fortified with ascorbic acid, more commonly referred to as vitamin C, the presence of a high concentration of ascorbic acid in urine is becoming relatively common. Further, high dosages of ascorbic acid are now advised by some people for disorders other than scurvy for which vitamin C is the prescribed thereapy. Thus, knowledge of a high concentration of ascorbic acid in urine specimens is necessary for the laboratory technician or physician to properly interpret the patient's urinalysis.

Most urine specimens contain ascorbic acid since the kidneys are the chief means of excreting surplus amounts of ascorbic acid from the body. However, since ascorbic acid in relatively high concentrations is known to interfere with many conventional tests associated with urinalysis, its unsuspected presence in high concentration can lead to many false conclusions pertaining to the results of the urinalysis.

In addition to testing urine specimens for ascorbic acid, a quick and simple test to check the concentration of ascorbic acid in foods is also desirable for quality control purposes in the food processing industry.

A dip and read test device consisting of paper impregnated with ammonium phosphomolybdate has been reported for determining the presence of ascorbic acid in fluids. However, the reported test device is inconvenient to prepare in that it requires a double impregnation of the bibulous paper, first with phosphomolybdic acid and then with an ammonium salt for the purpose of preparing ammonium phosphomolybdate and a separate drying step after each impregnation is necessary. In addition, the device is reported to be stable for only a few days. Also, to adapt the prior art device to testing urine specimens, it is first necessary to acidify the specimen to be tested with a strong mineral acid prior to testing in order to lower the pH of the specimen and thereby avoid urate interference.

The test composition of this invention is designed to overcome the stability and preparation problems encountered in the prior art and obviate the need to acidify the specimen to overcome the urate interference possibility commonly associated with testing urine by providing an integral test solution.

SUMMARY OF THE INVENTION

A test composition to determine the concentration of ascorbic acid in fluids comprising one or more phosphomolybdate salts and a soluble nitrate salt. The test composition may further include an organic acid to adapt the composition for urinalysis.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The test composition of this invention is a reagent formulated to determine the presence and concentration of ascorbic acid in fluids. Generally the composition includes an indicator and a stabilizing agent dissolved in solution. An organic acid may also be included in the solution to adapt the composition for urine analysis.

More specifically, the composition includes one or more phosphomolybdate salts and a neutral nitrate salt, each dissolved in an aqueous solution. A solid organic acid such as malonic acid, or the like, may be added to the foregoing solution to prevent possible urate interference when the fluid to be tested is urine.

The composition indicator is preferably one or more phosphomolybdate salts which provide a noticeable color change in the presence of ascorbic acid. This color change is known in the art to be caused by a reduction of the phosphomolybdate salt to molybdenum blue. It has been found that the indicator in the composition must be present in a concentration of at least 0.1 percent by weight and preferably from about 0.1 percent to about 0.6 percent by weight.

Sodium phosphomolybdate salts have been found to be acceptable for use as an indicator in the composition. It has been found that equal quantities of sodium 2-phospho-18-molybdate and sodium phospho-12-molybdate are preferred since such a combination provides both a rapid reaction in the presence of ascorbic acid and an intense color change.

To stabilize the above solution, from about 1.0 percent to 10 percent by weight of a neutral nitrate compound is added to the solution. Preferably, a concentration ranging from about 1.0 percent to about 3.9 percent by weight of the nitrate compound has been found effective. It has been found that nitrate salts, such as potassium or sodium nitrate, are acceptable for this purpose.

To adapt the above-described composition to testing urine specimens, it has been found that the addition of a suitable organic acid to the composition is recommended to avoid possible urate interference. The addition of from about 0.5 percent to about 3.0 percent by weight of an organic acid is recommended to lower the pH of the test specimen and thereby provide an integral test composition. It should be recognized by those skilled in the art that the addition of the acid enables the composition to be used for all types of testing, although its inclusion in the composition is only necessary to provide a test composition for testing samples where urate interference is possible. Malonic acid has been found to be the organic acid of choice since it is readily available, highly soluble, and does not react with the indicator incorporated into the composition.

The above-described composition is readily adaptable for use as a reagent for conducting spot tests for the presence and concentration of ascorbic acid in fluids to be analyzed. For the sake of brevity, the procedure for conducting spot tests is well known in the art and particular applications of the test composition of this invention to this type of test would prove to be verbose.

The above-described composition is also conducive for incorporation with a carrier for dip and read type of devices. For this purpose, an appropriate bibulous material or carrier is chosen, such as filter paper, wood, cloth or the like. The bibulous material is impregnated by saturating the material with the composition. The saturated material is then dried at approximately 90°C to about 130°C to evaporate or remove the aqueous vehicle of the composition. The dip and read test devices may then be cut to a workable and economical size and stored until needed for use. The composition may also be incorporated with polymeric semi-permeable membranes or matrices to form a paperless dip and read device.

To use the thusly-prepared test device, it is only necessary to dip the device into the fluid to be tested, remove the device therefrom, and note the color change, if any, apparent thereon. The thusly-prepared device may also be coated with a semi-permeable membrane such as ethyl cellulose to adapt the device for testing whole blood. As with the spot test, the presence of any ascorbic acid in the test fluid will cause the indicator present in the test composition or test device to turn a blue color. The intensity of the color is directly proportional to the concentration or amount of ascorbic acid present in the test sample. A color chart may be provided to facilitate interpretation of the test results.

The following examples will serve to illustrate the improved composition and the test device of this invention.

EXAMPLE I

To 100.0 ml. water, 0.2 gm. of sodium 2-phospho-18-molybdate, 0.2 gm. of sodium phospho-12-molybdate and 2.0 gm. of sodium nitrate were added and thoroughly mixed until dissolved. Five sheets of E&D No. 204 filter paper were then separately immersed into the above solution until saturated. The sheets were removed from the impregnating solution and placed in a forced air oven and dried at about 100°C for about 15 minutes.

The dried, impregnated sheets were observed to be off-white to light yellow in color. The sheets were then cut into strips and placed within moisture-proof, light-resistant bottles for testing or storage.

To test the thusly-prepared test devices, solutions were prepared containing 40 mg percent, 120 mg percent, 240 mg percent and 400 mg percent of ascorbic acid in water. A control containing only water was also tested.

The test devices were dipped into the above solutions and the following color changes in the test devices were observed after approximately 60 seconds:

Solution	Color
Control	no change
40 mg% of ascorbic acid	very light green
120 mg% of ascorbic acid	chartreuse
240 mg% of ascorbic acid	bluish-green
400 mg% of ascorbic acid	deep blue

EXAMPLE II

To 100.0 ml. of water, 0.2 gm. of sodium 2-phospho-18-molybdate, 0.2 gm. of sodium phospho-12-molybdate, 2.0 gm. of sodium nitrate and 2.0 gm. of malonic acid were added and thoroughly mixed. Test devices were prepared with this solution and tested as in Example I. Substantially the same results were observed.

Additionally, urine and serum samples containing unknown amounts of ascorbic acid were tested with the devices of Exam-ples I and II. Substantially the same results were observed in testing the serum samples with the devices of Examples I and II. In testing the urine specimens, it was noted that the color varied with the devices prepared according to the two examples. The devices prepared according to Example I changed to a deeper blue color indicating a higher concentration of ascorbic acid present in the specimens than did the devices of Example II.

The specimens were then conventionally assayed by titration with N-Bromosuccinimide as described in Analytical Chemistry 27:536-540, April, 1965. The analysis confirmed the results shown with the test devices containing malonic acid, whereas the devices prepared absent malonic acid indicated a higher concentration of ascorbic acid present in the urine than that which was actually found by assay. This indicates that the device absent malonic acid is susceptible to urate interference in urine causing an undesirably high and inaccurate reading.

EXAMPLE III

Test devices prepared in accordance with Examples I and II, only absent the sodium nitrate, were prepared. These devices and those of Examples I and II were placed in ovens set at 60° and 70°C. After 24 hours, the test devices were examined and no change was observed in the sodium nitrate treated devices while a color reaction was observed in the untreated devices.

Likewise, the test devices of Examples I and II, together with the devices prepared absent the sodium nitrate, were exposed to air at room temperature. Upon observation after exposure to air after 48 hours, the devices containing the sodium nitrate had not changed while the test devices lacking the sodium nitrate stabilizer had changed to a bluish-green color. The stabilized test devices were again observed at weekly intervals for four weeks and no change was observed.

An improved test composition and device for determining the concentration of ascorbic acid in fluids have thus been described which are both simple to use, accurate and stable. Additionally, the composition and test device are simple to prepare and use, the test device requiring only a single impregnation and thereby providing an economical test. Likewise to incorporate a compound to prevent urate interference provides an integral testing composition or device adaptable for testing urine.

What is claimed is:

1. A test composition to determine the presence and concentration of ascorbic acid in fluids, comprising:
an aqueous solution containing one or more sodium phosphomolybdate salts and a neutral nitrate salt.

2. A test composition as defined in claim **1** wherein the solution contains from about 0.1 percent to about 0.6 percent by weight of the phosphomolybdate salts.

3. A test composition as defined in claim **2** wherein said solution contains from about 1.0 percent to about 10.0 percent by weight of the said nitrate salt.

4. A test composition as defined in claim **3** wherein said nitrate salt is sodium nitrate.

5. A test device for determining the presence of ascorbic acid in fluids comprising a dry carrier material incorporated with the composition of claim **1.**

6. A test device as defined in claim **5** wherein said composition also contains a solid organic acid.

7. A test device as defined in claim **6** wherein said organic acid is malonic acid.

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