

# Review of methods for determining sorbitol in animal biological fluids

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**Abstract.** Article analyzes the main sorbitol detecting methods in animal biological fluids. Also it presents a brief methodology for conducting these studies. Respectively the main methods' advantages and disadvantages are outlined. Upon the conclusion several proposes were made for veterinary practitioners. For example, some prospects for using methods during laboratory examination.

## 1 Introduction

Sorbitol, or glucite ( $C_6H_{14}O_6$ ), is one of the most important compounds in animal biochemistry. It is a polyhydric alcohol found naturally in plants, especially fruits and seeds. Sorbitol is widely used in various physiological processes and plays a key role in maintaining the vital functions of organisms [1].

One of the most important aspects related to the value of sorbitol is its role in carbohydrate metabolism. Inside cells, sorbitol takes part in glycolysis and gluconeogenesis, the processes of synthesis and breakdown of carbohydrates. It can be used, albeit indirectly, as an energy source or converted into other important biomolecules such as amino acids and lipids.

Another important function of sorbitol in biochemical processes in animals is its role in supporting regenerative processes in cells. Sorbitol is able to prevent cell damage caused by free radicals due to its antioxidant properties. It protects cells from oxidative stress, prevents aging, and maintains the health and longevity of animal organisms [2].

Also, sorbitol, due to its high biotransformation associated with the hepatobiliary system, can act as a marker of the state of this system. In particular, its pharmacokinetic characteristics and clearance rates can be used for this purpose. However, the main problem in this scientific direction is limited information about methods for determining sorbitol in animal biological fluids [3].

## 2 Materials and methods

The search and processing of scientific publications were carried out according to H. Snyder's recommendations for writing review articles [4]. The bibliographic databases

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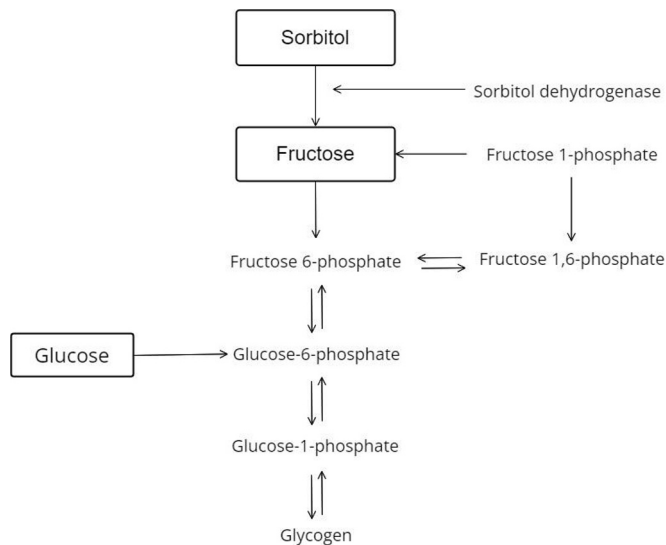
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(Elibrary, CyberLeninka, Pubmed, Scopus (Elsevier), and Web of Science (Clarivate)), in English and Russian, were searched for thematic publications using the keywords "clearance test with sorbitol", "methods for determining sorbitol", "determination of sorbitol in biological fluids» with further selection of the most cited ones. Articles published before 2013 were used only if they contained information critical to the topic that was not present in more recent publications. The figures in the article were made using Visual Paradigm online.

### 3 Results

The main route of sorbitol biotransformation in the body occurs through its oxidation. Sorbitol oxidation is carried out by sorbitol dehydrogenase, an enzyme present in various tissues of the animal body, especially in the liver and kidneys.

Sorbitol dehydrogenase reacts with sorbitol, turning it into fructose. This process is required to ensure further metabolism of sorbitol (Figure 1). Produced fructose can be used by the body for energy or converted into other metabolic compounds.



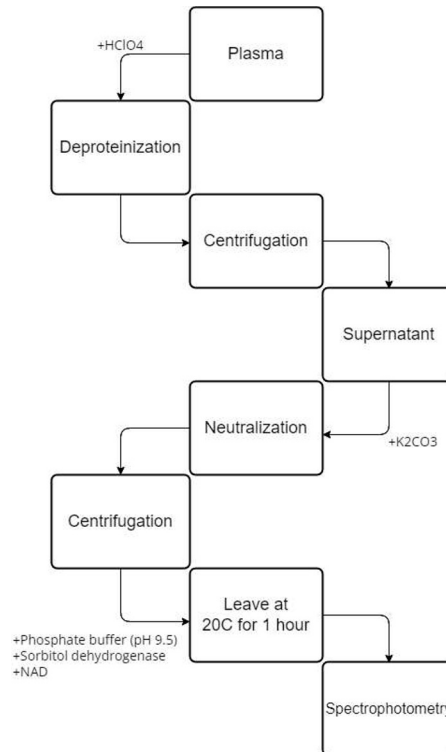
**Fig. 1.** Metabolism of sorbitol in the body.

The biotransformation of sorbitol also occurs in the intestines, where it can be hydrolyzed to fructose by fructosidase, an enzyme present in the intestinal mucosa. This process becomes especially important when consuming food containing sorbitol or its derivatives [5-6]. The determination of sorbitol in biological fluids is an important process in many scientific and medical studies [7]. There are several ways to determine sorbitol content, each of which has its own advantages and limitations.

One of the most common methods for sorbitol determination follows the principle of an enzymatic reaction. This method uses the enzymatic activity of sorbitol dehydrogenase, which reversibly converts sorbitol to fructose. This reaction releases NADPH (undenatured nicotinamide adenine dinucleotide phosphate), which can be detected by spectrophotometry. This method is precise and sensitive, but it requires specialized equipment and knowledge. There are several modifications to this method:

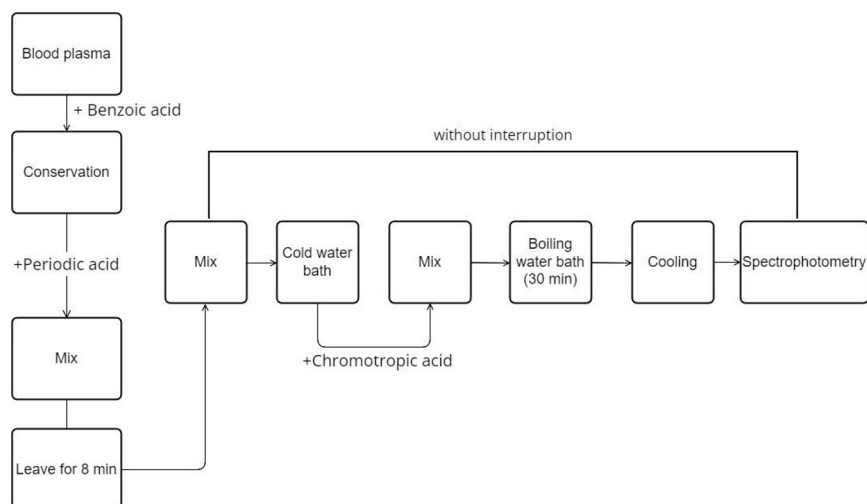
1) Determination of sorbitol in RBC with the enzymatic fluorometric method (M. Umeda et al.). According to the method, blood plasma must first be deproteinized with

perchloric acid. Next, the sample was centrifuged to obtain the supernatant and its subsequent neutralization with  $K_2CO_3$ . After this, a second centrifugation is performed, and phosphate buffer (pH 9.5), sorbitol dehydrogenase, and nicotinamide adenosine dinucleotide (NAD) are added to the supernatant. The resulting solution is set aside at 20 °C for 1 hour and then undergoes spectrophotometry with a pure buffer solution as a control (Figure 2). The main disadvantage of this method is the rather specific requirement for equipment (a fluorescence spectrophotometer) [8].



**Fig. 2.** Determination of sorbitol in RBC with the enzymatic fluorometric method (M. Umeda et al.).

2) There is also a Corcoran and Page method with modifications. The reagents for this method are: periodic acid (potassium periodate in sulfuric acid), tin chloride in hydrochloric acid, chromotropic acid in sulfuric acid, and sulfuric acid. The sample is initially preserved with benzoic acid. All reagents are added to the sample in a specific order. First, the periodic acid reagent is added, and the contents are immediately and thoroughly mixed with a stirring rod and left for 8 minutes. Subsequent steps of the procedure must be completed without interruption or delay. After 8 minutes, the tin chloride reagent is added, the reaction solution is mixed well again, and it is placed in a cold water bath. Then a chromotropic acid reagent is added, and the contents are mixed. The sample, with a stirring rod, is immersed in a boiling water bath for 30 minutes. After cooling, sulfuric acid is added, the contents are thoroughly mixed, and the stirring rod is removed. The resulting solution is read on a spectrophotometer against distilled water. With this method, the color and density of the solution are stable and remain unchanged for several hours (Figure 3). The main disadvantage of this method is the large number of steps required for determination. However, this method is one of the main working methods [9-10].



**Fig. 3.** Corcoran and Page method with modifications.

Another common method for sorbitol determination is the high-performance liquid chromatography (HPLC) method. This method is based on separating the test mixture into its components and determining the sorbitol content using a detector such as a spectrophotometer or an electrochemical detector. HPLC provides highly accurate and reproducible results but also requires specialized equipment.

In the scientific literature, you can find descriptions of the following method for determining sorbitol using thin-layer chromatography (TLC):

Plate. TLC plate with a silica gel layer.

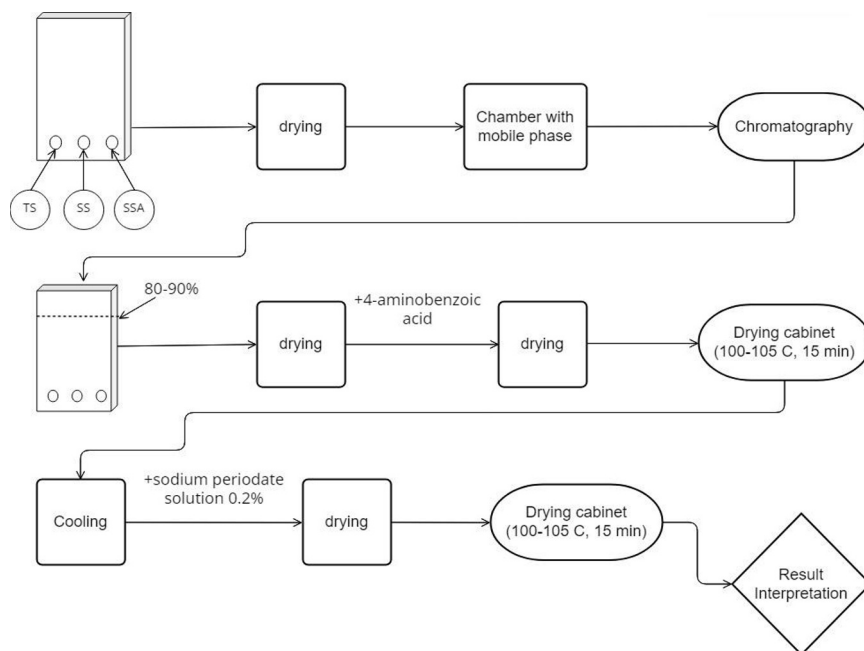
Mobile phase (MP). Water - ethyl acetate - propanol (10:20:70).

Test solution (TS). 25 mg of the test substance is placed in a 10 ml volumetric flask, dissolved in water, and the volume of the solution is adjusted to the mark with the same solvent.

Sorbitol standard sample solution (SS). Place 25 mg of a standard sorbitol sample into a 10 ml volumetric flask, dissolve it in water, and adjust the volume of the solution to the mark with the same solvent.

Solution for checking the separation ability of a chromatographic system (SSA). 25 mg of a standard sample of mannitol and 25 mg of a standard sample of sorbitol are placed in a 10 ml volumetric flask, dissolved in water, and the volume of the solution is adjusted to the mark with the same solvent.

2  $\mu$ l of the TS, SS, and SSA are applied to the starting line of the plate. The plate with the applied samples is air-dried, placed in a chamber with PF, and chromatographed using the ascending method. When the PF front has passed about 80–90% of the plate length from the starting line, it is removed from the chamber, dried until traces of solvents are removed, sprayed with a 4-aminobenzoic acid solution, dried in a stream of cold air until traces of acetone are removed, and kept in a drying cabinet at a temperature of 100–105 °C for 15 minutes. The plate is cooled at room temperature, sprayed with a 0.2% solution of sodium periodate, dried in a stream of cold air, kept in a drying cabinet at a temperature of 100–105 °C for 15 minutes, and viewed in visible light [11, 12] (Figure 4).



**Fig. 4.** Method for determining sorbitol using thin-layer chromatography (TLC).

## 4 Conclusion

Determining the level of sorbitol in biological fluids such as blood, urine, and saliva can be useful in diagnosing various conditions and diseases [13-16]. For example, an increased level of sorbitol may indicate a disorder in sugar metabolism or the presence of diabetes [17-20]. The level of sorbitol can also be associated with various pathologies, such as pathologies of the hepatobiliary system. Accordingly, its pharmacokinetic characteristics can be used to draw conclusions about the functional state of the hepatobiliary system.

## Acknowledgement

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