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Appraisal of Techniques, Investigation and Analysis of Vitamin (B7) Biotin

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Abstract

Biotin is also called vitamin B7, vitamin B8 or vitamin H. Biotin is a water soluble compound and is colorless in appearance. Overall eight different types of biotin exist but only Biotin-D occurs naturally with its complete vitamin activity. It is mainly synthesized by mold, algae, bacteria, yeast and some plant species. There are different methods used to find the vitamins in nutrients and samples. This article is composed of the comprehensive review of the competitive techniques and various methods for the assay of biotin. High-pressure liquid chromatographic (HPLC), microbiological analysis and HPLC producer are adopted for the determination of biotin. An optimal analytical condition of biotin determination was performed by the HPCL method. The statistical parameters of the HPLC methods were compared and reviewed with other determination.

Keywords

Biotin, Biotin-D, HPLC Method, Vitamin B8, Vitamin H, Biological Method, Microbiological Analysis

Subject Areas: **Pharmacology**

1. Introduction

Biotin, which is an essential vitamin, helps in the health of skin, nerve and digestive system, and assists in releasing energy and metabolism of fats, protein and carbohydrates [1]-[4]. It also helps in the formation of embryonic development [5]-[7]. It has wide function and effects on systemic processes and development [8] [9] assistance in immunity [10]. Biotin acts as prosthetic group of carboxylases; it is a hydro-soluble vitamin. Biotin also regulates gene expression unrelated to its role as carboxylase prosthetic group [11] [12]. Deficiency in the biotin may lead to the neurological disease and affect the skin and hair growths [13].

The glucose and carbohydrates are metabolized with the help of biotin [e]. Dakshinamurti *et al*. reported the

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evidence of the glucose metabolism and effects of biotin in the biotin deficient rats. The research carried out the Glucose Tolerance Test (GTT) curve in the biotin-deficient rats and significant results showed that the GTT curve is higher in the biotin-deficient rats than in the non-deficient rats [14]. The results indicate the reduction in the activity of hepatic glucokinase in the liver. It is responsible in glucose uptake in the liver. The effect of biotin on glucokinase is also observed in cultured rat hepatocytes and appears to be mediated through biotin-induced increases of cyclic GMP [14]. Later Chauhan *et al*. demonstrated the stimulatory effect of the biotin occurring at the level of transcription. This study helps to determine the role of biotin in the gene expression. The natural sources of biotin are fish, milk, egg yolks, liver and beans. Vitamin B7 (biotin) is also found in rice, wheat, nuts yeast, potatoes and cauliflower [15].

Maeda Y. *et al*. carried the research based on biotin deficiency in rats. The research concluded that biotin deficiency changes the activity and gene expression of urea cycle enzymes. For this experiment, Maeda Y. *et a*l. used rats which were sub-divided into two groups: biotin-deficient rats (BD rats) and biotin-supplemented rats (BS rats) (**Figure 1**). The plasma ammonia concentration was found significantly higher in the BD rats than in the BS rats. These results define that the deficiency in biotin may lower the activity of ornithine transcarbamylase in lever [16].

2. Methods of Analysis of Biotin B7

2.1. Liquid Chromatography (LC)

(LC) is a separation technique used for the determination of the amino acids, protein and vitamins, in which the mobile phase is a liquid. It can be carried out in column or a plane. Now a day's liquid chromatography generally consumes very small particles packing's and a relatively high pressure is referred to as high pressure liquid chromatography (HPLC) [17].

2.2. HPLC Operating Method

2.2.1. High Pressure Liquid Chromatography (HPLC) in Biotin

In HPLC the sample is highly forced by a mobile phase liquid at high pressure through a column that is packed with a stationary phase composed of irregularly or porous spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is divided into two different sub-classes based on the polarity of the mobile and stationary phases [17].

2.2.2. Assay Procedure

D. Scott Wilbur *et al*. carried out research to assess the relative binding of biotin derivatives with avidin and strept avidin for evaluation of biotin-Dye conjugates for use in an HPLC Assay. In this study HPLC is used to assess the relative association rats and dissociation rats of biotin derivative form avidin. In this experiment biotin dye conjugates were evaluated to determine their peak characteristics on two different size exclusion HPLC columns. Biotin-dye conjugates bound with avidin in the presence of equal quantity of biotin provided association rate relative to biotin has been studied. The percentage of biotin-Dye conjugates had tested 3× times slower than biotin. The biotin-cyanocobalamin was selected as best assay for HPLC. The biotin sarcosine-cyanocobalamin conjucate is more rapid dissociation rate than other biotin-Dye conjugates; this was conformed in this research via HPLC assay [18].

Patil Ashih *et al.* carried out a simple, sensitive and fast method using HPLC for the determination of Biotin, for the experiment UV-visible water2489 detector is attached with HPLC. The chromatic separation was achieved with a mobile phase containing water. The output calibration curve was linear with correlation coefficient of 0.99. The methods has shown consistence recovery of biotin and made this method specific, accurate and precise [19].

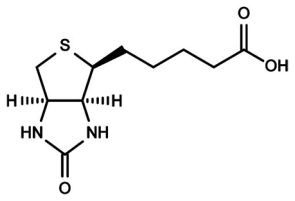

Figure 1. Structure of biotin.

Brain Kanagy worked on the quantitation of biotin. It compare the Quant*Tag*Biotin kit with the traditional avidin binding spectrophotometric 2-(4'-hydroxyazobenzene) benzoic acid (HABA) assay procedure. The protocol was followed to determine the biotin as given with the kit. The solution was prepared and incubated for 30 minutes. A mass spectroscopy of the unlabeled protein and biotinylated protein has been performed. The result concluded the single spike is determined for the unlabeled protein and multiple spikes for the biotinylated protein. The result showed the enzymatic digestion may reduce in the commercial biotin quantitation kits [20].

Biotin is repeatedly linked with protein and nucleic acid covalently. Robert H Batchelor *et al*. worked on the protein binding biotin based on the micro plate based high throughput fluorometric assay. The assay uses the Alexa fluor 488 dye tagged avidin with the quencher dye HABA. The experiment shows the HABA quenches with the fluorescence resonance energy transfer (FERT). The result shows that fluorescence intensity is directly proportional to the amount of biotin in the sample. The direct relation between the fluoresce emission and biotin help to determine the accurate amount of the biotin present in the sample [21].

Pranee Nandhasri *et al*. worked on HPLC in early 80's to determine the biotin in foods using HPLC. Pranee Nandhasri found the royal jelly (RJ) and bee larvae are rich in biotin and HPLC is quit reliable, sensitive and satisfactory analytical method for the determination of the biotin [22]. Later on it is found Biotin also found in natural product like fish, milk, and egg yolk [15]. HPLC is now widely used analytic method for the determination of the biotin from natural products [23].

1) Advantage of chromatographic techniques

One of the advantages of chromatographic and electrophoretic techniques is that they allocate the analyst the freedom to doggedness a complex mixture by diverse routes utilizing different partition mechanisms supported on the chemical physical and physical possessions of the solute mixture [24].

2) Disadvantage of HPLC and protein-binding assays

The disadvantages of these assays are their lack of bigotry between biotin and its metabolites combining protein binding assay and HPLC can both be able to resolve this predicament [25].

3) Microbiological assay procedure

Essential nutrients are found in plants and animals, these essential nutrients are important for the human beings. Bishnoi Kapil *et al*. studied the antibiotic and vitamins using various strains of microbes via micro biological array. The experiment shows the microbiological array methods is better than chemical methods. The experiment is based upon the compression of growth bacteria by measuring the vitamin concentration and known concentration. The known concentrations are the standard concentration of vitamin with known activity. The procedure is accurate, precise but it requires time to determine the concentration of the bacterial growth [26].

3. Result

The result is based on the work carried out by the different researchers and their experiments. The previous studies have shown that the microbiological assay and high performance liquid chromatography are widely used in the detection of the vitamins, amino acids and other nutrients. This review article demonstrates the comparison between the microbiological assay and HPLC assay to evaluate the biotin in food, human body and animals. The previous study has revealed that HPLC is the most reliable, detailed and précised results that provide consistence result. Among all these advantages over microbiological assay to determine the biotin, the HPLC is the quit fast and time saving method [27].

4. Discussion

Various methods and procedures of vitamin determination were carried out by microbiological assay and high pressure liquid chromatography method both analytical methods were widely used from extensive period of time. The enzymatic activity of the long term incubation may reduce the accuracy of the result. This appraise has shown microbiological assay procedure of the biotin analysis and determination is renounce time consuming and less precise [21]. Preparation of the sample is difficult to handle for such long period of time. While research work has shown that HPLC is the classical method for the biotin analysis in food and samples. Pharmaceutical and food industries have vast use of the fortitude of vitamins in their products. Time is one of the important features in every diligence to balance the demand and supply, HPLC is preferred over microbiological assay to reduce the time of determination of results. Previous studies have also shown that the HPLC is sensitive, accurate, reliable and analytical tool for the research and industries. Our research group has also done these types of reviews on different

topics [28]-[30].

5. Conclusion

Various methods and procedures for determination of vitamins and nutrients are used. Biotin is a specific vitamin helping in the metabolism of carbohydrates and protein. There are most widespread processes including microbiological assay and high pressure liquid chromatography technique. HPLC is nowadays the most competitive technique and classical method for biotin determination.

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