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A validated RP-HPLC method for estimation of hepatitis B surface antigen (HBsAg) in bulk preparation

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ABSTRACT

A Simple Selective, rapid, precise and accurate reverse phase high pressure liquid chromatographic method has been developed for the estimation of Hepatitis B surface antigen (HBsAg) in pharmaceutical formulation. The method was carried out on a Gracesmart C_{18} 5 micron (250 mm x 4.6mm i.d.) column with mobile phase consisting of 5 mmol potassium dihydrogen phosphate: Methanol (50: 50 v/v) and pH was unadjusted. The flow rate was kept 1.0 mL/min. Detection was carried out at 280 nm. The retention time of HBsAg was found to be 7.80 min. The developed method was validated for linearity, accuracy, precision (inter day and intra day), limit of detection and limit of quantitation.

Key words: Hepatitis B, RP-HPLC; HBsAg, Surface antigen.

INTRODUCTION

Hepatitis B vaccine (recombinant) contains purified major surface antigen of hepatitis B virus manufactured by recombinant DNA technology and adsorbed on aluminium hydroxide. The antigen thus produced assembles spontaneously into spherical particles of 20 nm in average diameter, containing non glycosylated hepatitis B surface antigen (HBsAg) polypeptide and a matrix consisting of phospholipids.[1-2]

Detailed survey of literature for estimation HBsAg antigen revealed several methods based on different techniques. Hepatitis B surface antigen (HBsAg) polypeptide can be assessed by size exclusion chromatography, sucrose centrifugation. Survey revealed a spectrophotometer method and an HPTLC method for estimation of HBsAg in dosage from. The Present work describes the development of RP- HPLC method, for estimation of HBsAg antigen and validation of method as per the ICH guidelines.[4]

EXPERIMENTAL

Instrumentation and Chromatographic Conditions

The LC system (Analytical, India) consisted of a solvent delivery module (ALC), analytical manual injector 2010 fitted with a 20 µL injection loop and a UV detector (ASPD). The column used was Grace Smart C_{18} 5micron {250 mm x 4.6 mm i.d.}. The mobile phase was prepared by mixing methanol: 5mmol potassium dihydrogen phosphate buffer in the ratio 50: 50, v/v; and the pH was unadjusted. The flow rate was kept at 1 mL min^{-1}. The detection wavelength was set to 280 nm and the operation was performed at ambient temperature. Operation, data acquisition and analysis were performed using Analchrom software.

These chromatographic conditions were developed by following a series of experiments in an effort to elute HBsAg at a retention time that is suitable for analysis with better peak shape.

Reagents and Chemicals

A reference standard of HBsAg was kindly provided by Dr. K.S. Jaganathan, Center for Drug Delivery Research, Tokyo University of Science, Chiba, Tokyo, Japan. Methanol was of HPLC grade (Rankem, Delhi), and potassium dihydrogen phosphate buffer (S. D. fine Chem., Mumbai) were analytical reagent grade. HPLC grade water was used wherever required.

Method Development[3,5-6]

Different mobile phases containing methanol, water and acetonitrile in different proportions were tried and finally Methanol: 5mmol potassium dihydrogen phosphate buffer in the ratio 50: 50, v/v; and the pH was unadjusted, was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for HBsAg.

Preparation of Standard Solution

Standard stock solutions of strength 1.0 mg/ml of HBsAg was prepared separately using methanol. Using Standard stock solution , different dilutions of HBsAg (1,2,4,8,16 & 24 µg/mL) were prepared in mobile phase .

Calibration Curve

Linearity of the system was investigated by serially diluting the stock solution to get concentrations in the range of 1 µg/mL to 24 µg/mL for HBsAg. An aliquot (20 µL) was injected using mobile phase as eluent.

Method Validation

As per the ICH guidelines[4], the method validation parameters checked were linearity, accuracy, precision, limit of detection and limit of quantitation[4].

Linearity and Range

The result of the method was found to be linear in the concentration range of 1 to 24 µg/ml and the coefficient of correlation was found to be 0.9999.

Accuracy and Precision

The accuracy of the method was determined by calculating percentage bias of three concentrations (2, 4, 8 µg/mL) from calibration curve, the accuracy was found in the range of -0.35 to 7.6%.

The precision of the method was demonstrated by inter day and intra day studies, 3 repeated injections of standard and sample solutions were made in a day and the response factor of drug peaks and percentage RSD were calculated and found to be not more than 1.144%. In the inter day variation studies. 3 repeated injections of standard and sample solution were made on 3 consecutive days and response factor of drugs peaks and percentage RSD for HBsAg was calculated and found to be not more than 0.94%. The data obtained indicates that the developed RP-HPLC method is precise.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyze that gives the measurable response. LOD was calculated using the following formula[4].

The LOD for HBsAg was found to be 13 ng/mL. The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula[4].

$$\text{LOD} = \frac{3.3 \times \text{Standard deviation}}{\text{Slope of calibration curve}}$$

The LOQ was 40 ng/mL for HBsAG.

RESULT AND DISCUSSION

The proposed method was found to be simple and linear in the concentration range of 1 to 24 µg/mL for HBsAg antigen. The method was found to be accurate and precise as indicate by recovery studies and % RSD not more than 1.14. Moreover, LOD and LOQ for HBsAg were found to be 13 ng/mL and 40 ng/mL, respectively, indicating the method is specific and sensitive.

CONCLUSION

The proposed RP-HPLC method for the estimation of HBsAg in bulk was found to be sensitive, accurate, precise, simple and rapid. Hence, the present RP-HPLC method may be used for routine analysis of the raw materials and formulations.

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