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Research Article

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Isolation of Protein From Dilute Solution by Chromatographic Method

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Abstract

High Pressure Liquid Chromatography (HPLC) is one of such sophisticated techniques by means of which we can do quantitative as well as qualitative measurement of different types of samples at a very low concentration even in the order of pictogram and nanogram level. The technology is an important one particularly in the field of Pharmaceutical Technology. Chromatography technique effects the separation of two or more component in a mixture. Our goal is to develop a unique method so that specific compound as mentioned can be identified in a single run from BSA protein qualitatively as well as quantitative from a BSA dilute solution. A Rheodyne model 7125 six port injection valve fitted with a 20 microlitre sample loop and a Novapak C18 column (150 mm x 39 mm, waters, USA) packed with 5 micrometer particles were used. The column was fitted with a Guard column (5 cm x 4.6 mm) packed with the same packing material as in Novapak column. Single protein BSA can be easily be enriched or separated with a optimum chromatographic condition, at a flow rate of 0.8ml/min., run time 8min, injection volume 20µl, mobile phase 0.9% NaCl + 10mM Tris buffer, pH 7.4. As a result BSA (Bovine serum albumin) produced a peak at 1.406min with the area 66944 and the peak height was 6291. Comparing with the standard it can easily conclude that the peak was only for BSA sample.

Keywords: BSA std, BSA dilute solution, Chromatographic method.

1.INTRODUCTION

High Pressure Liquid Chromatography was developed in mid-1970's, and quickly improved with the development of column packing materials and additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography environment. It has applications also for local drug delivery to the stomach and proximal small intestines. The controlled GRD forms may be achieved by the mechanisms of (1) muco-adhesion, (2) flotation, (3) sedimentation, (4) expansion modified shape Systems, (5) simultaneous administration of pharmacological agent. This review focuses on the principal mechanism of floatation to achieve gastric retention. allowed for improved separation between very similar compounds with the development of column technology methods are developed to analyze different types of compounds including the trace elements including a variety of biological samples. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniquees. Computers and automation added to the curve -nience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge. The past decade has been seen a vast undertaking in the development of micro-column,andother specialized columns. The dimensi -ons of the HPLC columns and internal diameter are varied to a great extent with respect to need analysis. Now a days micro-columns, or capillary columns, Fig 1. Standard Curve of BSA solution.

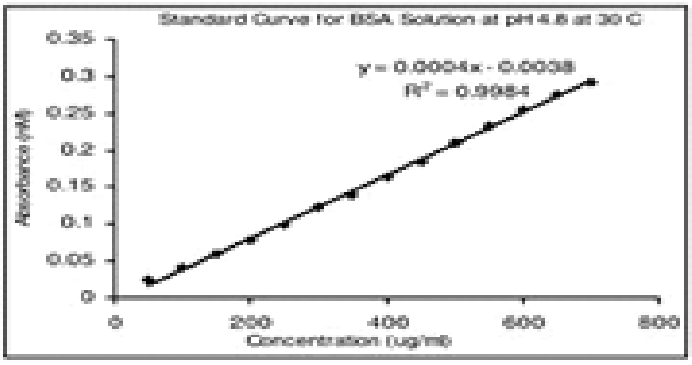

Fig 1. Standard Curve of BSA solution.

ranges from 3micrometer to 200 micrometer are regularly used for detecting a very short time. Bovine serum albumin (BSA) is a byproduct of cattle industry. It is a single polypeptide chain consisting of about 600 amino acid residues and carbohydrates.At pH 1-7 it contains 17 intrachain disulphide linkages with sulfhydryl group. It has numerous biochemical applications including (Enzyme-Linked Immunosorbent Assay, ELISA), blots test. It is also

used as a nutrient in cell and microbial culture. This does not affect other enzymes that do not need it for stabilization. BSA is used because of its stability, no ill effect in many biochemical reactions, and its low cost. Our goal is to develop a unique method so that specific compound as mentioned can be identified in a single run fromBSA protein qualitatively as well as quantitative from a BSA dilute solution.

2. METHODS AND MATERIALS

2.1. RP-LC Analysis of Whey

Bovine Serum Albumin (BSA), Sodium chloride (0.9%w/v) both were purchased from Sigma Aldrich (USA). A Rheodyne model 7125 six port injection valve fitted with a 20 Microlitre sample loop and a Novapak C18 column (150 mm x 39 mm, waters, USA) packed with 5 micrometer particles were used. The column was fitted with a Guard column (5 cm x 4.6 mm) packed with the same packing material as in Novapak column. The detector system was waters 996 PDA detector and the analysis was performed use in millennium-32 upgrade software (waters USA). The whole system was Supplied By waters USA. UV-Spectrophoto-meter, Model- SHIMADU UV-1700, with UV PROBE software was supplied by SHIMADU. Digital weighing balance by SWISSER. Digital pH meter supplied by, MODEL NO 112.

2.2. Preparation of Standard Curve of BSA

Required quantity of powdered BSA was weighed and dissolved in double distilled water and then suitably diluted to get the desired concentration in the range of 50-800 mcg/ml. The optical density (OD) was observed using UV-spectrophotometer at λ_{max} of 280 nm. The OD values were plotted against concentration to obtain a standard curve. The standard curve was the average of five observations. Later quantification of BSA protein in the samples, collected during experiments, was performed by UV spectrophotometer at λ_{max} 280 nm.

3. RESULTS AND DISCUSSIONS

In the present study whole protein was estimated by UV-spectrophotometer and major parts of datawere obtained by UV analysis which is not adequate to analyse individual proteins of whey. As variation of composition of whey from natural source is observed, it is important to develop a reliable and fast analytical method to analyze protein in the dilute solution. There is already an abundance of literature concerning BSA protein analysis. Methods include gel electrophoresis, chromatographic and electrophoresis, capillary electrophoresis and immunochemical detection. By far the most used method for BSA protein analysis is

liquid chromatography. No one method is suitable enough for all the BSA protein and the method of choice is usually based on one or two particular proteins of interest. In particular high-

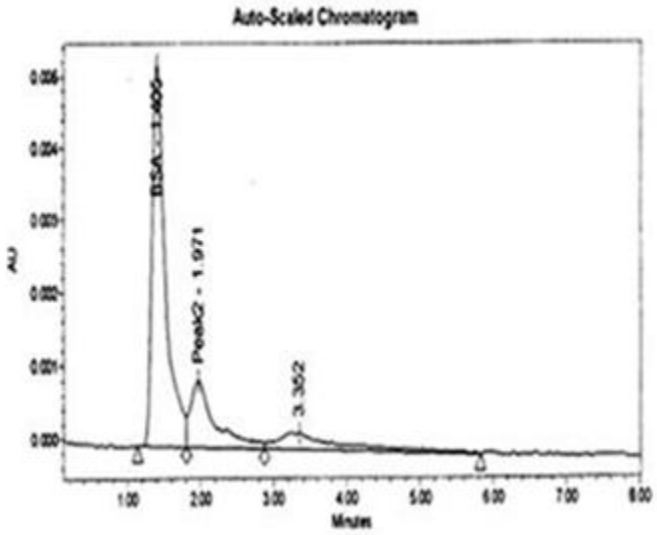

Figure 2: Chomatogram of Unknown Sample at Chromatographic Conditions

performance liquid chromatography (HPLC) has become one of the main techniques in the dairy industry as it combines versatility, short analysis time and high resolution with increasingly sophisticated automation and superior column performance. Manji and is coworkers (1985) reported HPLC method for the analysis of mixture of pure proteins (β-Lg-A, β-Lg-B, α- La and serum albumin) by anionic exchange chromatography. Total time of analysis per sample was 40 min. Parris and his coworkers (1991) developed a method (RP-HPLC) for the quantification of denaturized whey protein. Analysis of protein took long time (1.5 h). A suitable analytical method is necessary to separate bovine serum albumin (BSA), to estimate these in protein of dilute solution within short period. In the present study a new method has been developed and validated . Typical RP-HPLC chromatograms of BSA, sample is shown in Figure 2. Retention time of BSA is 1.406. No interference peak was observed in the RP-HPLC profiles for blank sample. Elution was monitored at wavelength of 280 nm to enable detection of BSA, α-La and β-Lg. Detection at this wavelength also had the advantage that it improved the sensitivity of response as well as minimized the impurities that was eluted within 280nm. From the standard curve it is very clear that the first peak is due to BSA sample. The second and the third peaks are from unknown sample may presented in the dilute solution of BSA sample.

4. CONCLUSION

Quantification of a material can be made precisely if suitable assay technique is adopted. A new HPLC method was developed and applied to the present studies. BSA fractionated in the HPLC column and results were satisfactory. A reverse phase HPLC method was presented for the determination of BSA in dilute solution as unknown sample. The method was found rapid, reliable and sensitive. The described method may have wide application in the routine analysis of BSA in dairy industries.

5. Acknowledgement

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