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# APPLICATION NOTE

# Capture of Glycoproteins by Aminophenyl Boronate Affinity Chromatography

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## Introduction

Glycoproteins constitute a wide range of proteins, defined by having a carbohydrate motif as an inherent part of their structure. Some of these have been developed as therapeutic agents such as erythropoetin, others exist as impurities in bioprocesses. In order to purify or remove these proteins at a commercial scale, chromatographic separation technology must invariably be employed.

A diverse variety of chromatographic media can be employed for such commercial manufacture of therapeutic biologicals, all of which respond in one way or another to the unique set of characteristics inherent to the protein of interest.

Here we illustrate the use of immobilized aminophenyl boronate for capture of glycoproteins. Aminophenyl boronate is a unique chromatographic separations tool because it exploits the presence of 1,2 and 1,3 cis-diol groups by the transient formation of a covalent bond to its boric acid group. Since such bonds are formed with carbohydrate groups, glycoproteins may be captured and purified using this technique. The aminophenyl boronate-glycoprotein covalent bond can be subsequently disrupted to elute the protein of interest with a pH change or by competitive elution using a sugar such as sorbitol.

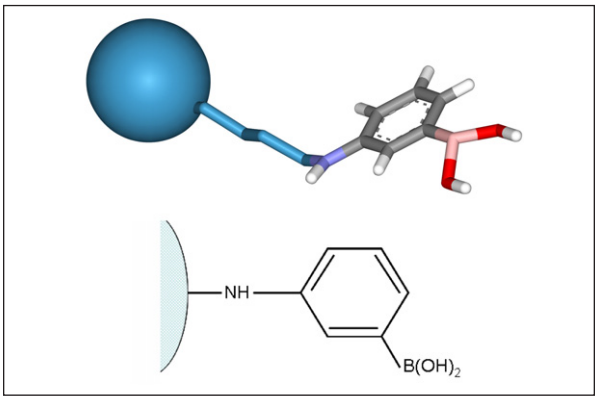


Figure 1: Chemical structure and schematic of aminophenyl boronate ligand attached to an agarose bead.

| Examples of Affinity Chromatography Using Aminophenyl Boronate | |
|---|---|
| ***Target Molecule*** | ***Examples*** |
| Proteins | Erythropoietin (EPO), Membrane Glycoproteins, Serum Proteins - Fetuin, Transferrin |
| Carbohydrates | Arabinose, fructose, galactose, inositol, lactose, sorbitol |
| Nucleotides/ Nucleosides | Adenosine, deoxyadenosine, cytidine, guanosine, thymidine, uridine |
| Nucleic Acids | Oligonuleotides and RNA |

This application note describes the separation of a yeast glycoprotein impurity from a non-glycosylated protein, and the capture of fetuin.

Fetuin is a serum glycoprotein widely distributed in mammals. Bovine fetuin contains up to 30% carbohydrate and has a molecular mass of 48 KDa. Also of note is its ability to promote cellular growth. It is therefore often used in cell culture and might be considered a common impurity in manufacturing processes where serum is employed to support cell growth. Fetuin is also regularly used as a model protein to represent the properties of glycoproteins, and has been used here to illustrate binding to aminophenyl boronate.

## Method

| Chromatography Conditions for Yeast Glycoprotein | |
|---|---|
| Column Height | 7.4 cm |
| Column Volume | 5.8 mL |
| Linear Flow Rate | 50 cm $hr^{-1}$ |
| Equilibration & Wash Buffer | 100 mM Na Phosphate, pH 8.0 |
| Elution | 20 mM Na Acetate, pH 4.0 |
| Sanitisation | 0.5 NaOH |
| Fractions were collected and tested for the presence of glycoprotein using a Sigma Glycoprotein Detection Kit. | |

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| Chromatography Conditions for Purification of Fetuin | |
|---|---|
| Column Height | 4.0 cm |
| Column Volume | 3.1 mL |
| Linear Flow Rate | 50 cm $hr^{-1}$ |
| Equilibration & Wash Buffer | 50 mM Na Phosphate, pH 8.0 |
| Elution | As equilibration with sorbitol gradient 0 - 200mM |
| Sanitisation | 1M NaOH |

## Results

Removal of yeast glycoprotein from non-glycosylated protein.

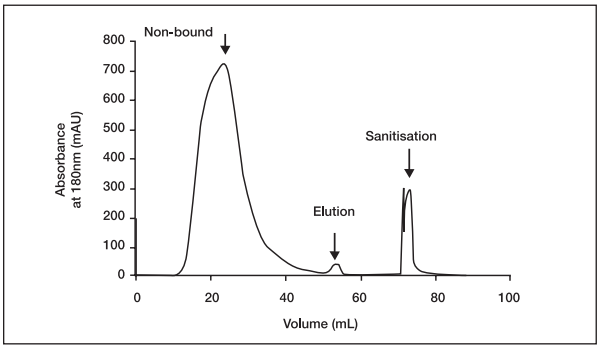


Figure 2: Chromatographic trace of non-bound and bound protein on aminophenyl boronate A6XL.

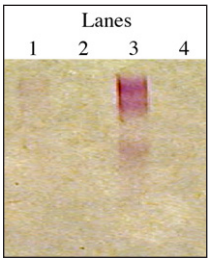


Figure 3: SDS-PAGE gel stained with Glycoprotein Detection Kit to identify position of glycoprotein applied and eluted from Aminophenyl Boronate A6XL.

Lane 1: load material,
Lane 2: unbound protein,
Lane 3: eluted yeast glycoprotein,
Lane 4: sanitised material.

Capture and sorbitol elution of serum glycoprotein, fetuin.

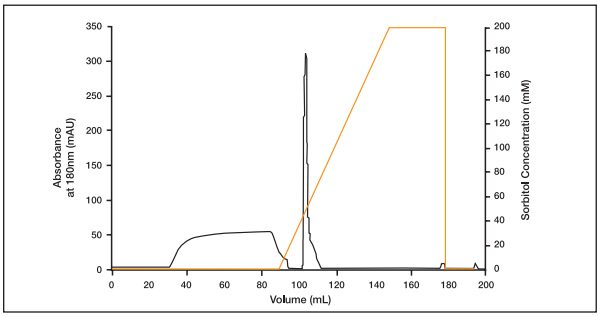


Figure 4: Chromatographic trace of fetuin applied to aminophenyl boronate A6XL and eluted using a sorbitol concentration gradient.

## Discussion

The use of immobilized aminophenyl boronate can be applied for the capture and purification of molecules containing chemical motifs such as 1,2 or 1,3 cis-diol groups, 1,2 hydroxy acids, and 1,3 hydroxylamines. These are typically found in the carbohydrate functionality of glycoproteins as well as a diverse range of biological entities.

These two experiments illustrate the application of aminophenyl boronate in the following ways:

- the separation of glycan-free protein from glycoprotein
- the use of a pH gradient to release bound protein from the media
- the use of sorbitol to competitively elute bound protein from the media.

In terms of commercial-scale manufacturing, Aminophenyl Boronate A6XL can therefore be applied to a range of uses, including the purification of glycosylated recombinant proteins from mammalian cell culture such as IgG, glycosylated proteins such as erythropoietin, or removal of glycosylated impurities in expressions systems such as recombinant yeast. The purification of glycated blood or plasma proteins, and the removal of impurities derived from such sources can also be accomplished.


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