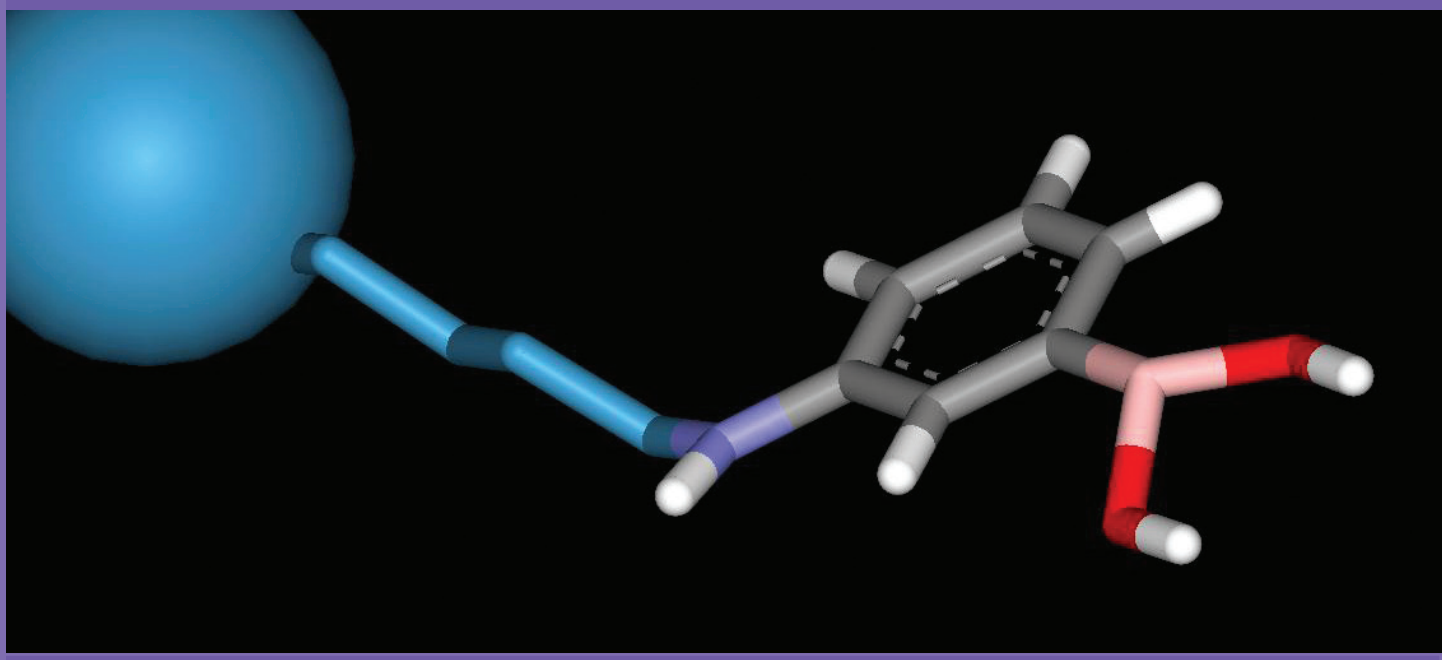


Aminophenylboronate

Aminophenylboronate P6XL
Aminophenylboronate A6XL

Aminophenylboronate affinity adsorbents are used for purification of a diverse range of macromolecules which possess 1,2- diols, 1,3-diols, 1,2-hydroxy acids and 1,2- hydroxylamine groups. These functionalities are present in glycoproteins, carbohydrates, nucleic acids (nucleosides, nucleotides and RNA's), and polyphenols (catechols, flavonoids).

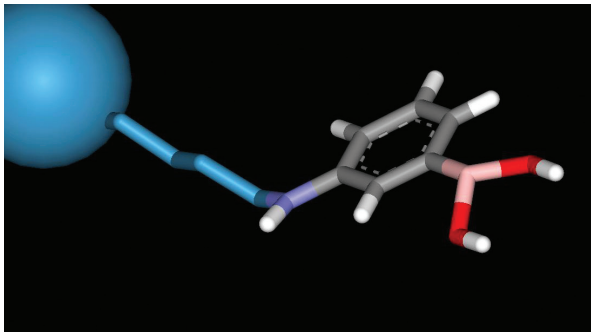
www.astreabioseparations.com

Search: Astrea Bioseparations

Astrea Bioseparations
Purity by Design

Our Technology

Aminophenylboronate Agarose affinity adsorbents are particularly suited to the purification of glycoproteins, or the removal of glycoprotein and carbohydrate impurities from non-glycosylated molecules. These adsorbents can also be used for the purification and removal of certain enzymes such as proteases and hydrolases where the boronic acid group has affinity for the active site.



The chemically stable affinity ligand is bonded to 6% cross-linked agarose beads to produce a highly robust adsorbent which is resistant to concentrated sodium hydroxide and suitable for use in downstream purification process applications and incorporation into diagnostic assays.

Key Features

- Affinity purification and removal of glycoproteins and glycans
- High purity m-aminophenylboronic acid ligand
- Ligand binds selectively to carbohydrate groups with cis-diols
- High dynamic binding capacity
- Robust, long life adsorbent
- Sanitisable with NaOH allowing multiple cycles
- Highly reproducible batch-to-batch manufacture to ISO 9001 standard
- Supported with comprehensive Regulatory Support Files
- Multiple end users in regulated bio-pharmaceutical manufacturing and medical diagnostics

Advantages of Aminophenylboronate Agarose Adsorbents:

Optimised product:

All of Astrea's Aminophenylboronate affinity products have been optimised for use in various bioseparation applications. All products share the same robust ligand and coupling chemistry.

Different Support matrix options:

Aminophenylboronate products are available based on two different beaded agarose materials. **Aminophenylboronate P6XL** utilises Astrea's proprietary Purabead® P6XL near-monodisperse® agarose beads to produce a highly robust cross-linked agarose beads. Purabead products have very uniform particle size which enables reproducible column packing and high flow rates at low back-pressure. Consequently Aminophenylboronate P6XL is the adsorbent of choice for bioprocess applications. **Aminophenylboronate A6XL** is Astrea's original product with a wider particle size distribution. This product is better suited to small scale or diagnostic applications.

Adsorbent Stability:

Astrea's Aminophenylboronate adsorbents can be subjected to pH's in the range 2.0 – 14.0 without significant ligand loss. This enables routine use of 0.5 M NaOH for column cleaning and sanitisation.

p-Aminobenzamidine

pH Stability

Our Aminophenylboronate adsorbents are stable across the pH range 3 -13 (continuous exposure) and compatible with intermittent exposure to pH 14.0.

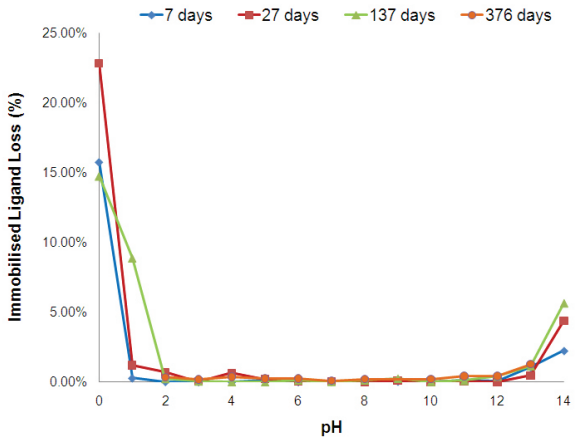


Figure 1: *pH stability profile for Aminophenylboronate A6XL incubated in a range of test solutions from pH 0.0 (1 M HCl) to pH 14.0 (1 M NaOH) at ambient room temperature. Samples of supernatant were collected and assayed for the presence of ligand at time intervals ranging from 1 week to 1 year continuous exposure.*

Flow rates

The uniform particle size of Aminophenylboronate P6XL enables operation of this adsorbent at high linear flow rates. Operational flow rates up to 500 cm/h may be used, though flow rates during column loading and elution are typically in the range 50 – 200 cm/h.

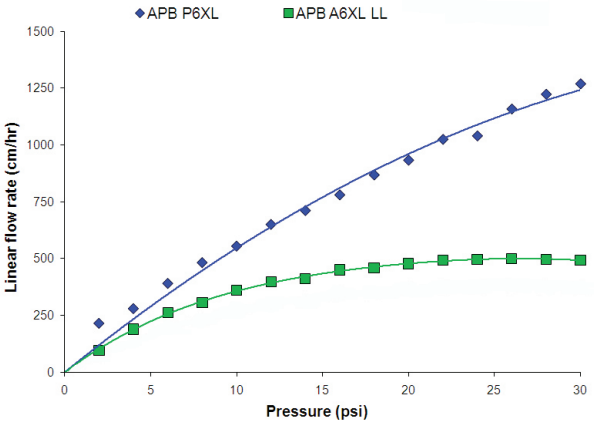


Figure 2: *Pressure-flow curve for Aminophenylboronate P6XL (3.2 cm dia. x 15 cm bed length).*

Binding Capacities

Binding capacities of Aminophenylboronate P6XL/A6XL are in the range 10 – 20 µmol/g for glycoproteins and up to 30 µmol/g for carbohydrates.

Operating Conditions

Binding of glycoproteins to Aminophenylboronate adsorbents is optimal at low to medium ionic strength (10 – 300 mM) and in mildly alkaline pH conditions (pH range 8.0 – 9.5). Buffers prepared using phosphate; borate and glycine are recommended for column equilibration. Flow rates of between 50 – 150 cm/h are recommended for binding. Adsorbent binding capacity is dependent on the nature of the sample and typically it is in the range of 10 – 20 µmol/g for glycoproteins which should be attainable without excessive process development.

Elution of the bound protein from Aminophenylboronate adsorbents is achieved either by using a low pH buffer or by the use of a competing diol. Elution by reduction of pH can be achieved using 0.1 M glycine/HCl or 0.1 M acetate buffer, pH 4.0. In the case of acid labile proteins, elution can be performed with a neutral pH buffer using a suitable competing diol such as 20 – 200 mM sorbitol.

Step	Condition
Equilibration/ Packing buffer	50 mM sodium phosphate, pH 9.0 buffer
Protein preparation	Prepare protein in equilibration buffer. Pre-filter at 0.45 µm or less.
Operating flow rate	50 - 200 cm h^{-1}
Elution	20 – 200 mM sorbitol or 0.1 M glycine/HCl or 0.1 M acetate buffer, pH 4.0
CIP	0.5 – 1.0 M sodium hydroxide
Re-equilibration	Equilibration buffer
Sanitisation	0.5 M NaOH
Storage	20% Ethanol

Application Examples

Purification of Fetuin with Aminophenylboronate P6XL:

Fetuin is a major 59 kDa glycoprotein in the serum of fetal calves containing three N-linked and O-linked carbohydrate side chains. Its purification from crude commercial preparation of fetuin on Aminophenylboronate Agarose P6XL column is illustrated.

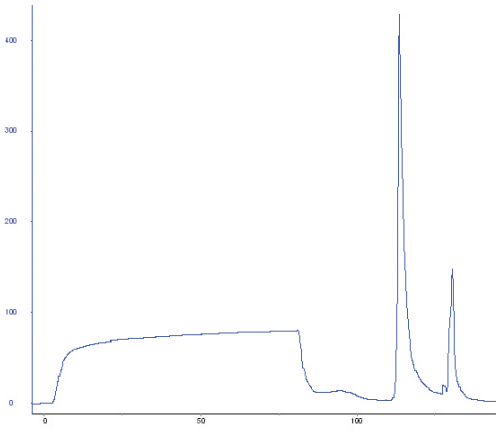


Figure 3: *Column chromatography of Fetuin.*

Column Height: *4 cm*
Column Volume: *3.1 ml*
Linear Flow Rate: *50 cm/h*
Equilbration & wash buffer: *50 mM sodium phosphate, pH 9.0*
Elution: *Sorbitol gradient (0 – 200mM)*
Sanitisation : *1M NaOH*

Capture of recombinant glycoproteins:

Aminophenylboronate adsorbents are particularly useful for the capture and purification of glycoproteins. Figure 4a demonstrates a classic affinity purification chromatogram with a large flow-through and a small, discrete elution peak. Full target glycoprotein recovery was attained after 50 mM sorbitol elution.

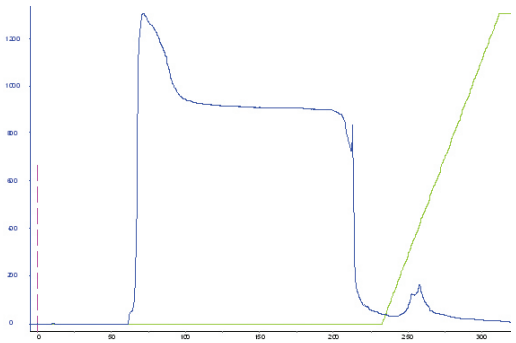


Figure 4a: *Capture of a recombinant glycoprotein expressed in a mammalian cell culture using Aminophenylboronate A6XL.*

Column: *(1.0 cm dia. x 7.4 cm bed height)*
Equilibration wash / buffer: *50 mM glycime.NaOH pH 9.0*
Elution: *50 mM phosphate buffer with a 0 - 200 mM sorbitol gradient*
Sanitisation: *0.5 M sodium hydroxide*
Flow rate: *200 cm/h*

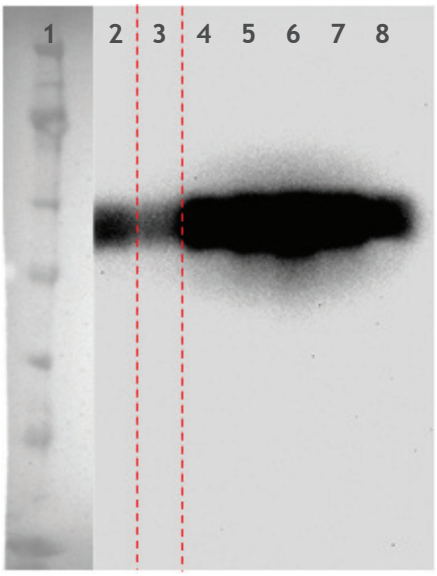


Figure 4b: *Western blot of chromatography fractions from the capture and recovery of glycoproteins using Aminophenylboronate A6XL.*

Lane 1: *Molecular weight marker*

Lane 2: *Sample loaded onto Aminophenylboronate*

Lane 3: *Flow-through pool from the column*

Lanes 4 - 8: Gradient elution fractions

Regulatory Support

Astrea's Aminophenylboronate Agarose adsorbents are supported by regulatory support files.

Bulk Manufacture

Astrea Bioseparations can provide Aminophenylboronate Agarose adsorbents in volumes sufficient to meet the requirements of the largest biomanufacturing processes. Single batches of up to 275 litres are produced in Astrea's controlled environment manufacturing facility under an ISO 9001 quality system.

Technical Support

Astrea Bioseparations has comprehensive knowledge of the properties and applications of Aminophenylboronate Agarose adsorbents We can provide full support for the development of new applications and their implementation for biopharmaceutical manufacture.

Properties of Aminophenylboronate P6XL	
Ligand:	m-Aminophenylboronic acid
Matrix:	PuraBead® P6XL (6% near- monodisperse cross-linked agarose).
Particle size:	100 ± 10 µm
Binding capacity:	Up to 30 mg sorbitol /ml
Operational Flow rate:	Up to 500 cm/h
Operating pressure:	Up to 3 bar (45 psi)
Operating pH:	pH 2 - 14
pH stability:	Long term (3 months) pH 3 - 13
Chemical stability:	All commonly used aqueous buffers and co-solvents.
Sanitisation:	0.5 - 1.0 M sodium hydroxide, 25°C
Storage:	24% ethanol / 76% 0.1 M NaCl

Ordering Information

For small orders up to 500 mL, please visit our webshop.

We also offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes.

Adsorbent Gel Slurry Aminophenylboronate P6XL

Pack Size	Product Code
25 mL	3355-00025
100 mL	3355-00100
500 mL	3355-00500
1,000 mL	3355-01000

Adsorbent Gel Slurry Aminophenylboronate A6XL

Pack Size	Product Code
25 mL	0355-00025
100 mL	0355-00100
500 mL	0355-00500
1,000 mL	0355-01000

Pre-Packed Columns

Astrea offer a range of pre-packed columns from a 1 mL and 5 mL column kits and 5 mL and 50 mL EvolveR columns for research and development as well as a range of up to 6.28 L GMP ready columns for pilot to process scale manufacturing.

For more details please visit our website or for more information on this or any other supply related matters please do not hesitate to contact us.

Contact Us:

With sales and support offices in North America and Europe, R&D facilities in Cambridge, UK and manufacturing facilities located on the Isle of Man, British Isles and in Joliette, QC, Canada we are able to meet your needs and support your application wherever you are.

Sales and Technical Support

Please contact us for further sales or technical support information regarding any of our innovative biochromatography products and services.

Horizon Park, Barton Road,
Comberton, Cambridge,
CB23 7AJ, UK

Tel: +44 (0) 1223 433 800

Sales:	sales@astrea-bio.com
Technical support:	techsupport@astrea-bio.com
Quality support:	quality@astrea-bio.com
Website:	www.astreabioseparations.com

Aminophenylboronate Agarose - V3

Astrea Bioseparations