

## APPLICATION NOTE

# Purification of Human Serum Albumin from Whole Plasma using Mimetic Blue 1 A6XL

### Introduction

A purification procedure involving the use of a Mimetic Blue 1 A6XL affinity chromatography resin for the purification of human serum albumin (HSA) from whole plasma is outlined below.

### Experimental Conditions

A 4ml Mimetic Blue 1 A6XL affinity chromatography column (Prometic Biosciences Ltd, UK) was loaded with 2 ml of pooled plasma, pH 6.0 (65mg protein) at a flow rate of 0.33 ml/min. Elution was undertaken using 50 mM sodium phosphate/2.0 M NaCl buffer, pH 8.0.

### Results

HSA bound to the Mimetic Blue 1 A6XL at pH 6.0 and was eluted using 50 mM sodium phosphate/2.0 M NaCl, pH 8.0 (Fig 1).

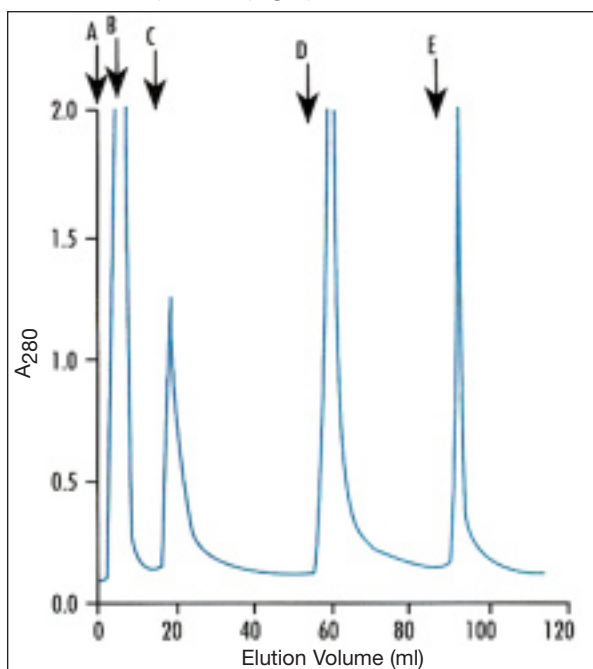


Fig 1. FPLC trace of the purification of HSA from whole plasma using Mimetic Blue 1 A6XL. (A) load; (B) Flow-through; (C) eluted contaminants, eluted using 50 mM sodium phosphate, pH 8.0; (D) HSA (>96% pure) & (E) residual contaminants brought off in the sanitisation (1 M NaOH).

### Analysis of repeated HSA purification from human plasma using Mimetic Blue 1 A6XL

Human plasma was diluted 1:1 with 25 mM MOPS buffer, pH 6.0 adjusted to pH 6.0 with 1 M HCl and filtered. At the beginning of each cycle, the column was equilibrated with 6 ml of 25 mM MOPS buffer, pH 6.0 and the column loaded with 1 ml of diluted plasma. After washing with 5 ml of equilibration buffer, bound HSA was eluted with 5 ml of 50 mM sodium phosphate buffer, pH 8.0 containing 1 M potassium chloride. After every fifth cycle 5 ml of 1 M sodium hydroxide was flushed down the column to regenerate it. Eluted HSA was collected every tenth cycle and analysed by absorbance at 280 nm and, at every fiftieth cycle, by SDS-PAGE. Column backpressure was also recorded every tenth cycle. A constant flow rate of 1 ml/min (300ml/h-1cm<sup>2</sup>) was used throughout the experiment.

### Results

Over a period of 300 cycles, negligible increase in column backpressure was observed (Fig 2). Similarly, the operational column capacity was hardly affected decreasing from 7.0mg/ml to 6.7 mg/ml (Fig 2). The elution peak profile was unaltered over the cycles (Fig 3) and the purity of the eluted HSA was constant throughout (Fig 4).

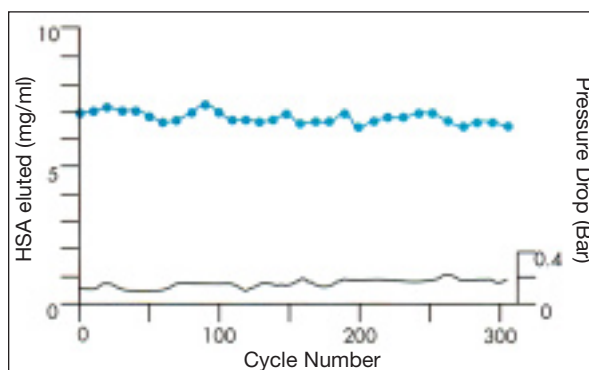


Fig 2. Working column capacity and pressure drop as a function of cycle number.

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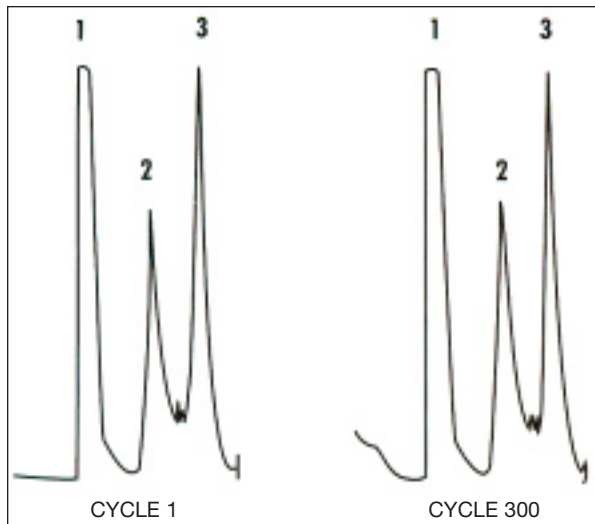


Fig 3. Purification of HSA on Mimetic Blue 1 A6XL: cycle 1 & cycle 300.

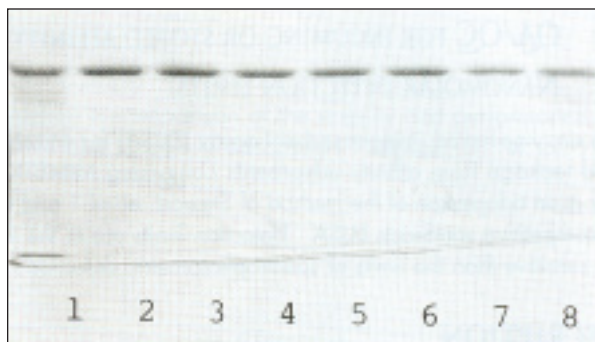


Fig 4. SDS-PAGE analysis of albumin purity at the function of number of purification cycles.

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