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Fast and robust purification of antibodies from human serum with a new monolithic protein A column

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SUMMARY

The use of antibodies is becoming more and more important. Therewith the need for fast and robust methods for their purification and columns with a long-life time is growing. In this application we describe a fast and robust protein A affinity chromatography method with a new monolithic column. Antibodies were successfully purified in less than 1.5 minutes with a flowrate of 5 mL/min highlighting the advantages of this column and method.

INTRODUCTION

Antibodies have a fundamental importance in the field of biotechnology and in the pharmaceutical industry. Besides their application for a variety of research tasks, they are essential components in the diagnosis and treatment of diseases. One of the most common methods to purify antibodies is a protein A affinity chromatography purification which can be very time-consuming. This capture step is often performed with a fast protein liquid chromatography (FPLC) system and an FPLC column. Monolithic columns are made of a continuous piece of a solid material. They are extremely permeable and offer a high efficiency that is even compatible with high flow rates [1]. This characteristic next to their extreme robustness to withstand high pressures makes monolithic columns an ideal candidate for fast chromatography methods.

RESULTS

The antibodies from 100 µL human serum were purified with a monolithic protein A column with a flow rate of 5 mL/min and a total run time of 1.5 minutes. The chromatogram is shown in **Fig. 1**. Peak A depicts the flow-through of unbound proteins. The antibodies were eluted with buffer B (Peak B) and collected. The concentration of the purified antibodies was determined, and a yield of 0.43 mg calculated. Additionally, SDS-PAGE was performed to check for impurities and analyze the purity of the collected samples. The protein bands of antibody heavy chain (HC) and light chain (LC) are visible at 48 kDa and 25 kDa in the SDS-PAGE (**Fig. 2**). Only minor contaminations are visible. The purification of antibodies from human serum was repeated 200 times under the same conditions (**Fig. 3**). The overlay of the repetitions shows no decrease in peak area and a standard deviation of 2.67 % confirming an overall good reproducibility of the runs.

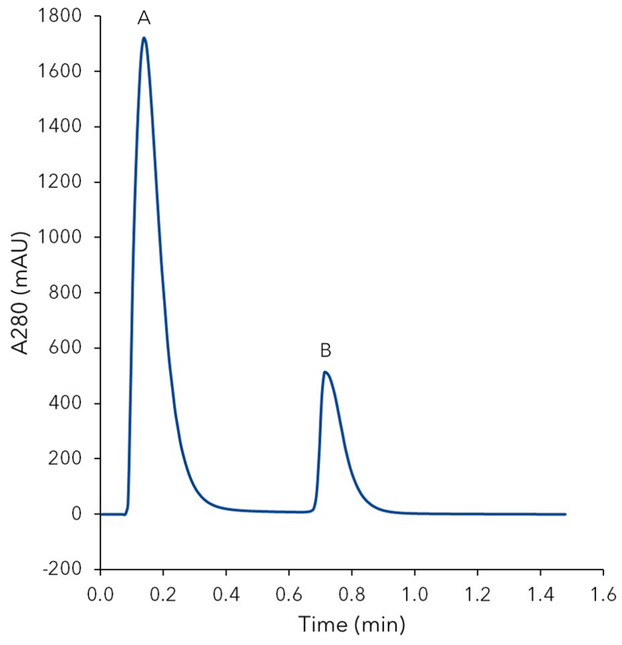

Fig. 1 Chromatogram of the IgG purification from human serum, detection at 280 nm UV signal, A) flow through of unbound protein; B) elution peak of IgG

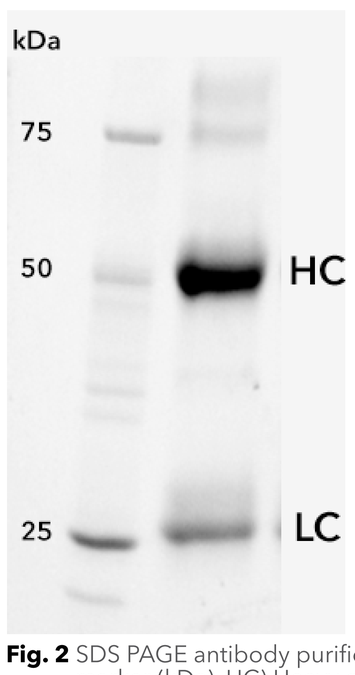

Fig. 2 SDS PAGE antibody purification, marker (kDa), HC) Heavy chain, LC) Light chain

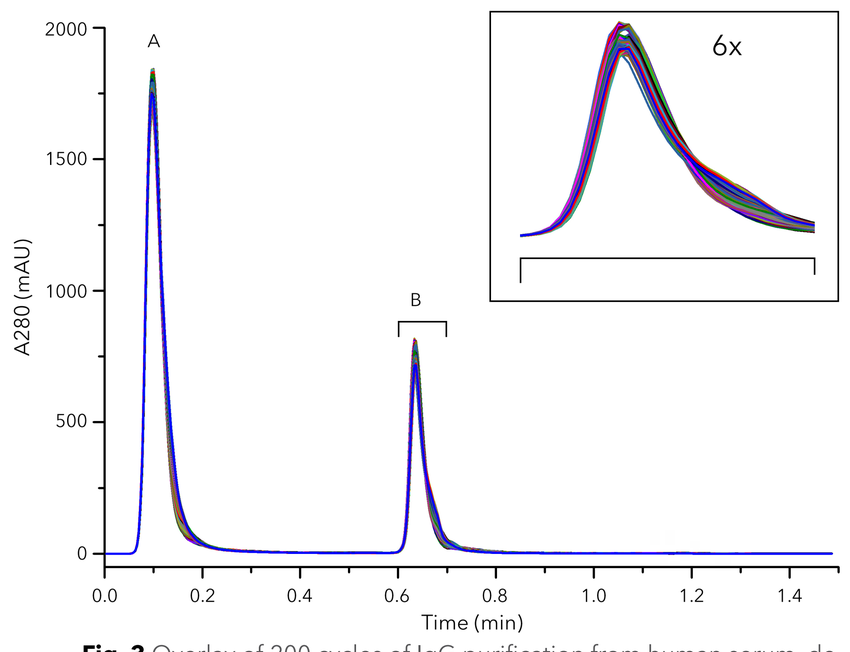

Fig. 3 Overlay of 200 cycles of IgG purification from human serum, detection at 280 nm, 6-fold zoom-in of peak B (0.6-0.7 min), A) flow through of unbound protein; B) elution peak of IgG

MATERIALS AND METHODS

An AZURA® Bio purification system consisting of an AZURA P 6.1L HPG metal-free pump, AZURA ASM 2.1L assistant module with feed pump and two 6 port/3 channel injection valves, an AZURA MWD 2.1L multi wavelength detector with semi-preparative biocompatible 3 mm, 2 µL flow cell cartridge; AZURA CM 2.1 conductivity monitor and a fraction collector was used. The monolithic protein A column (0.75 mL column volume [CV]) was equilibrated with a minimum of 10 CV buffer A (100 mM phosphate buffer pH 7.4 + 150 mm NaCl) at 5 mL/min. 100 µL of human serum was injected and the column was washed with buffer A. IgG was eluted with the elution buffer B (100 mM phosphate buffer pH 2.2 + 150 mm NaCl) and collected with the fraction collector. The UV signal was measured at 280 nm and a conductivity signal was recorded. The concentration of the IgG fraction from each individual run was determined with a NANODROP 2000 and the samples were analyzed for purity by SDS-PAGE.

CONCLUSION

Human antibodies were successfully purified with a new protein A affinity chromatography monolithic column on a KNAUER AZURA® Bio purification system. The purification was very fast with a run time of 1.5 minutes. Quality and quantity of the purified antibody was excellent. The columns have a very long-life time and reproducibly over 200 cycles. These columns are ideal for a fast and reliable purification procedure of antibodies.

REFERENCES

[1] Monolithic columns in high-performance liquid chromatography. Guiochon G. J Chromatogr A. 2007 Oct 19;1168(1-2):101-68; discussion 100. Epub 2007 Jun 3. Review. PMID: 17640660

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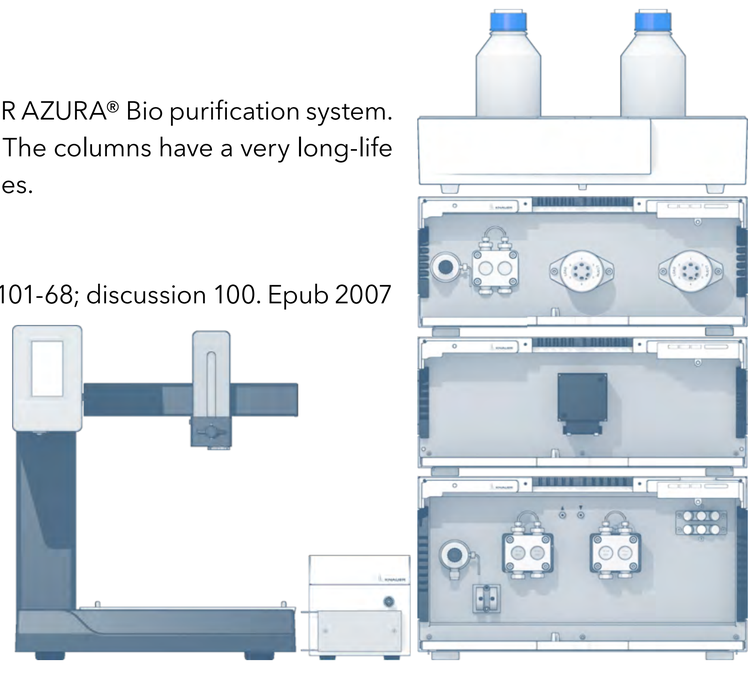

Additional information

VBS0068

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ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

Eluent A	100 mM sodium phosphate buffer pH 7.4 + 150 mm NaCl		
Eluent B	100 mM sodium phosphate buffer pH 2.2 + 150 mm NaCl		
Gradient	Time [min]	% A	% B
	0.0-0.5	100	0
	0.5-1	0	100
	1.5-3	100	0
Flow rate	5 mL/min	System pressure	-
Column temperature	RT	Run time	1.5 min
Injection volume	100 µL	Injection mode	Full loop
Detection UV	280 nm	Data rate	2 Hz

Tab. A2 System configuration & data

Instrument	Description	Article No.
Pump	AZURA P 6.1L High Pressure Pump with 50 ml pump head, Ceramic, without Degasser	APH68FB
Detector	AZURA MWD 2.1L	ADB01
Flow cell	Semi-preparative KNAUER PressureProof UV Flow Cell Cartridge	AMB18
Conductivity monitor	AZURA CM 2.1S	ADG30
Assistant	AZURA ASM 2.1L L: AZURA pump P4.1S, 50 ml Ti M: 6-Port/3-channel valve head (PEEK) R: 6-Port/3-channel valve head (PEEK)	AYBHECEC
Column	Monolithic protein A coumn	
Fraction collector	Foxy R1	A59100
Software	Purity Chrom 3D Option	A2654

RELATED KNAUER APPLICATIONS

VBS0063 - Automated two - step purification of mouse antibody IgG1 with AZURA Bio LC Lab system

VBS0064 - Comparison of IgG purification by two different protein A media

VBS0065 - Separation of two model proteins with ion exchange chromatography

VBS0067 - Automated two-step purification of 6xHis-tagged GFP with AZURA Bio LC

VBS0066 - Fast and sensitive size exclusion chromatography of IgG antibody

VBS0069 - Purification of Sulfhydryl Oxidase