

Monolithic anion-exchange chromatography as a process step for the preparation of a high-quality immunoglobulin G-based product with relevance for emerging virus outbreaks



Tihana Kurtović^{1,2}, Adela Štimac^{1,2}, Sanja Mateljak Lukačević^{1,2}, Saša Kazazić³, Beata Halassy^{1,2}

Centar Izvrsnosti
Virusnu imunologiju i Cjepiva

Center of excellence
for
Virus Immunology and Vaccines

¹ University of Zagreb, Centre for Research and Knowledge Transfer in Biotechnology, Rockefellerova 10, 10000 Zagreb, Croatia

² Center of Excellence for Virus Immunology and Vaccines, CERVirVac, Rockefellerova 10, 10000 Zagreb, Croatia

³ Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia



INTRODUCTION

Today, when epidemics of infectious diseases are occurring more often, and spreading faster and further than ever, there is a great need for more sustainable production approach that would be goal-oriented towards assuring easily available plasma-derived immunoglobulin of therapeutic relevance. Unlike other therapies, convalescent plasma becomes available as soon there are survivors, without any need for further development.¹⁻³

Here, we propose a refinement strategy for the preparation of ready-to-use immunoglobulin G (IgG) from COVID-19 convalescent plasma (CCP), achieved through simplification and reduction of processing steps. The manufacturing procedure was guided by the idea of persistent keeping of IgG molecules in solution, so that protection of their native structure could be assured. Our production approach was efficiently performed by sequence of only three steps: caprylic acid (CA) precipitation of unwanted proteins, diafiltration of immunoglobulin-enriched fraction and CIM[®] monolithic anion-exchange chromatography under conditions that allow adsorption of IgA, IgM and other residual impurities exclusively, while at the same time enable passing of IgG through the stationary phase without binding. The recovery and purity of IgG was monitored in every processing step, enabling accurate estimation of the procedure's cost-effectiveness.

WORKFLOW

Plasma downstream processing

anti-SARS-CoV-2 convalescent plasma

Caprylic acid (CA) precipitation

removal of non-IgG proteins

Diafiltration

removal of remaining CA

CIM[®] monolithic anion-exchange chromatography

removal of IgA and IgM

Pure IgG

Assessment methods

Electrophoresis
LC-MS/MS
Protein concentration estimation
SEC and RP chromatography
ELISA

Immunoglobulin sample was loaded (2 mL per run) to the pre-equilibrated CIM QA disk ($V = 0.34$ mL) with 20 mM sodium acetate buffer, pH 5.0 at a flow rate of 1 mL min^{-1} on an ÄKTA chromatography system. The absorbance was monitored at 280 nm. After collecting the flow-through fraction, the bound components were eluted from the column with binding buffer containing 1 M NaCl. During elution step flow rate was 2 mL min^{-1} .

RESULTS

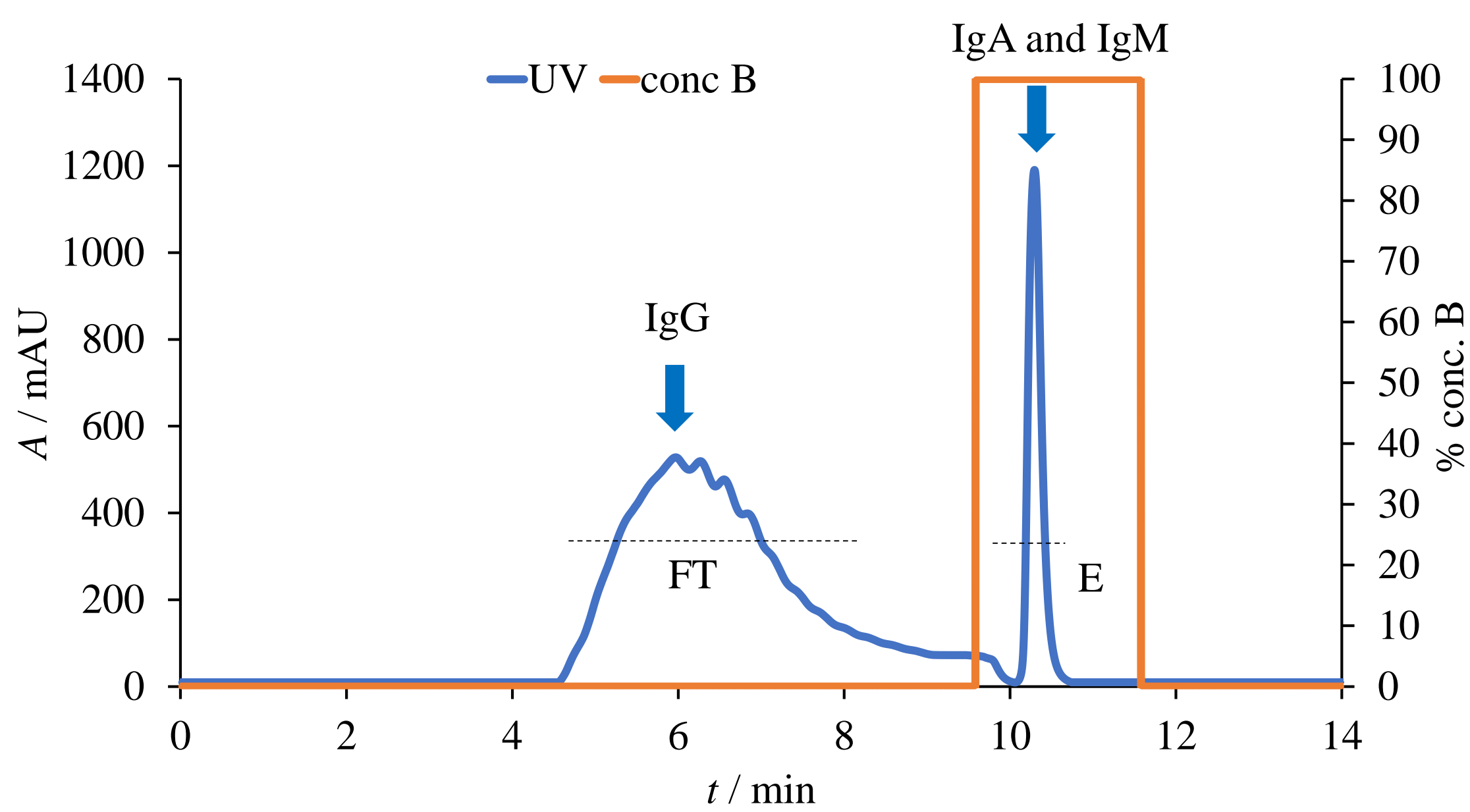


Figure 1. Final polishing of the IgG preparation. Anion-exchange chromatography of immunoglobulin sample on CIM QA disk ($V = 0.34$ mL) with 20 mM sodium acetate buffer, pH 5.0. After collecting the flow-through fraction (IgG), the bound components (IgA and IgM) were eluted from the column with binding buffer containing 1 M NaCl.

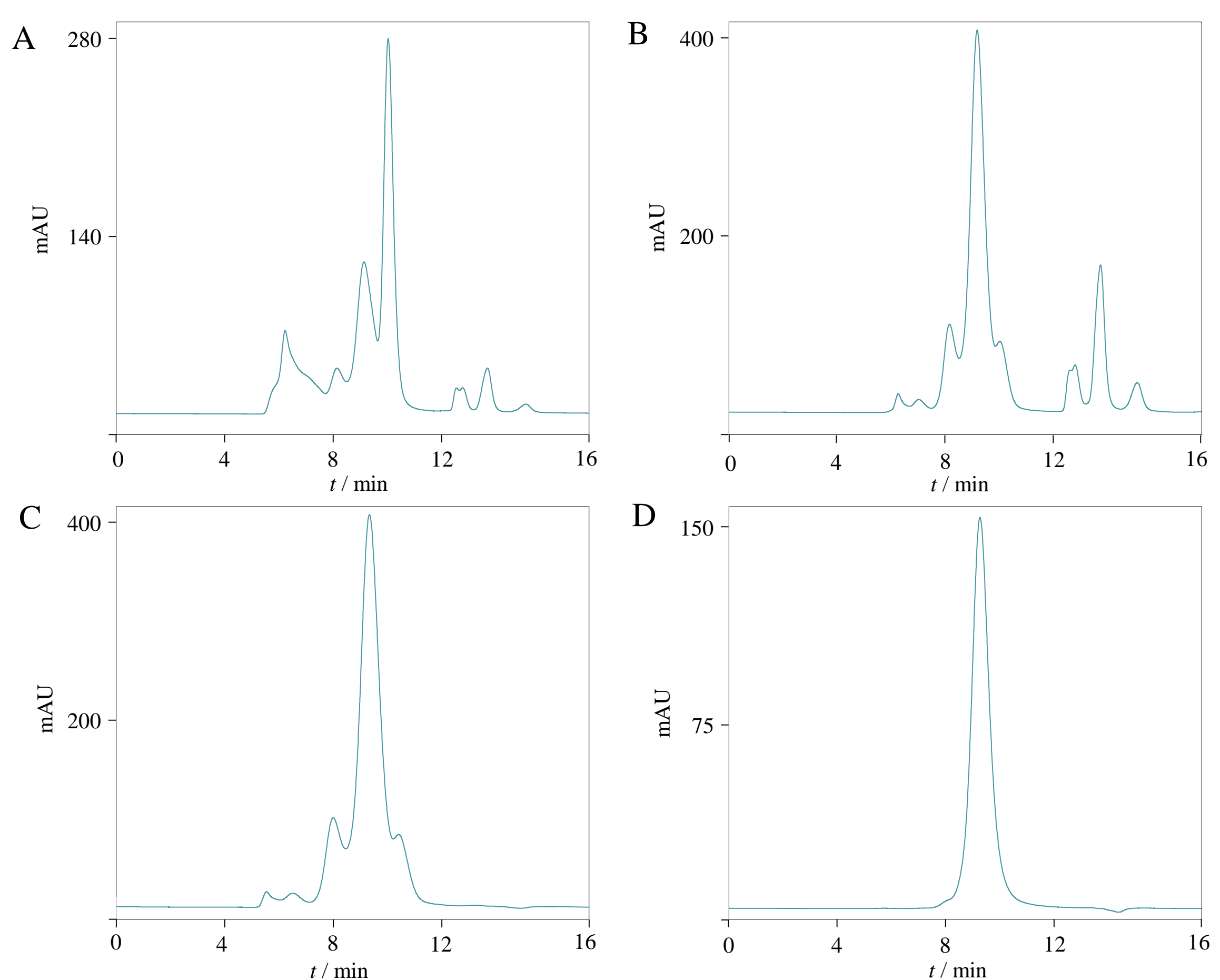


Figure 2. Purity profiling by size-exclusion chromatography. Analysis was performed on TSK-Gel G3000SWXL column with 0.1 M phosphate-sulphate running buffer, pH 6.6, at a flow rate of 1 mL min^{-1} . A) Heat-treated anti-SARS-CoV-2 convalescent plasma, B) IgG fraction obtained by CA precipitation, C) IgG fraction after diafiltration using a 100 kDa membrane, D) Pure IgG; flow-through fraction from anion-exchange chromatography, E) Multi-detector SEC chromatogram of pure IgG - refractive index (red), right angle light scattering (green), low angle light scattering (black), viscometer (blue), ultra violet (purple).

Table 1. Share of IgG, IgA and IgM isotypes within total protein or immunoglobulin content in the intermediate fractions and the final product of developed downstream processing protocol, together with their recoveries in every fractionation step. Samples were submitted to at least three total protein/immunoglobulin concentration measurements for purity and yield calculations. Results are given as mean from eight independently performed COVID-19 convalescent plasma (CCP) fractionations \pm 95% CI.

		IgG	IgA	IgM
Caprylic acid precipitation	Share in total proteins [%]	72.5 ± 4.9	9.8 ± 2.6	4.8 ± 3.2
	Share in IgGs [%]	83.2 ± 2.2	11.8 ± 3.3	5.2 ± 3.4
	Recovery in relation to CCP [%]	84.6 ± 6.7	77.4 ± 11.2	42.6 ± 7.4
Diafiltration	Share in total proteins [%]	79.2 ± 7.6	10.7 ± 3.0	4.9 ± 3.5
	Share in IgGs [%]	83.6 ± 2.6	12.9 ± 4.0	4.8 ± 3.3
	Recovery in relation to CCP [%]	81.8 ± 8.0	73.8 ± 9.6	40.1 ± 7.5
CIM[®] monolithic anion-exchange chromatography	Recovery in relation to previous step [%]	96.5 ± 3.2	95.9 ± 4.5	83.0 ± 12.1
	Share in total proteins (IgGs) [%]	99.2 ± 0.1	0.7 ± 0.1	$0.6 \times 10^{-3} \pm 0.4 \times 10^{-3}$
	Recovery in relation to CCP [%]	75.1 ± 1.8	4.1 ± 1.5	0.2 ± 0.1
	Recovery in relation to previous step [%]	93.0 ± 7.1	5.6 ± 2.0	1.0 ± 0.8

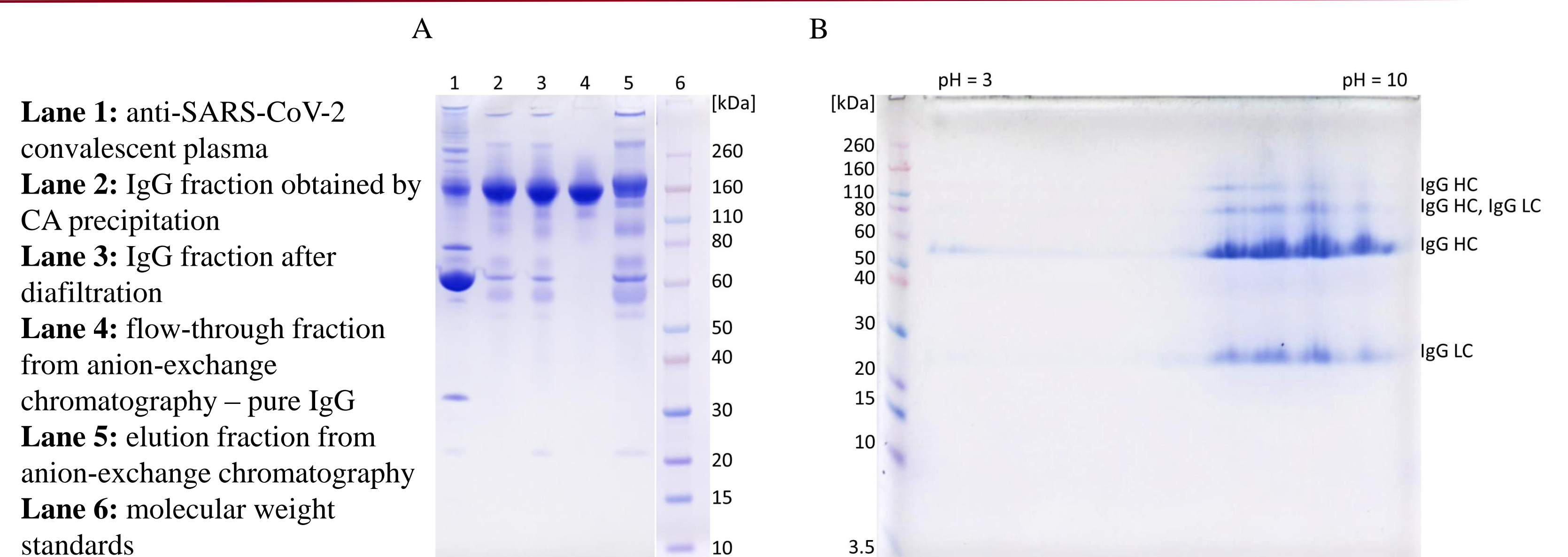


Figure 3. A) SDS-PAGE analysis of representative samples from purification process. B) 2D gel electrophoresis of the final product. In the first dimension IgG ($m = 250$ μg) was focused using IPG strip under denaturing conditions (linear pH 3-10). Prior second dimension IPG strip was reduced, alkylated and loaded to a 4-12% gel. Proteins were detected with CBB R250 and identified by MS/MS analysis (LC = light chain, HC = heavy chain). Molecular mass markers are at left side.

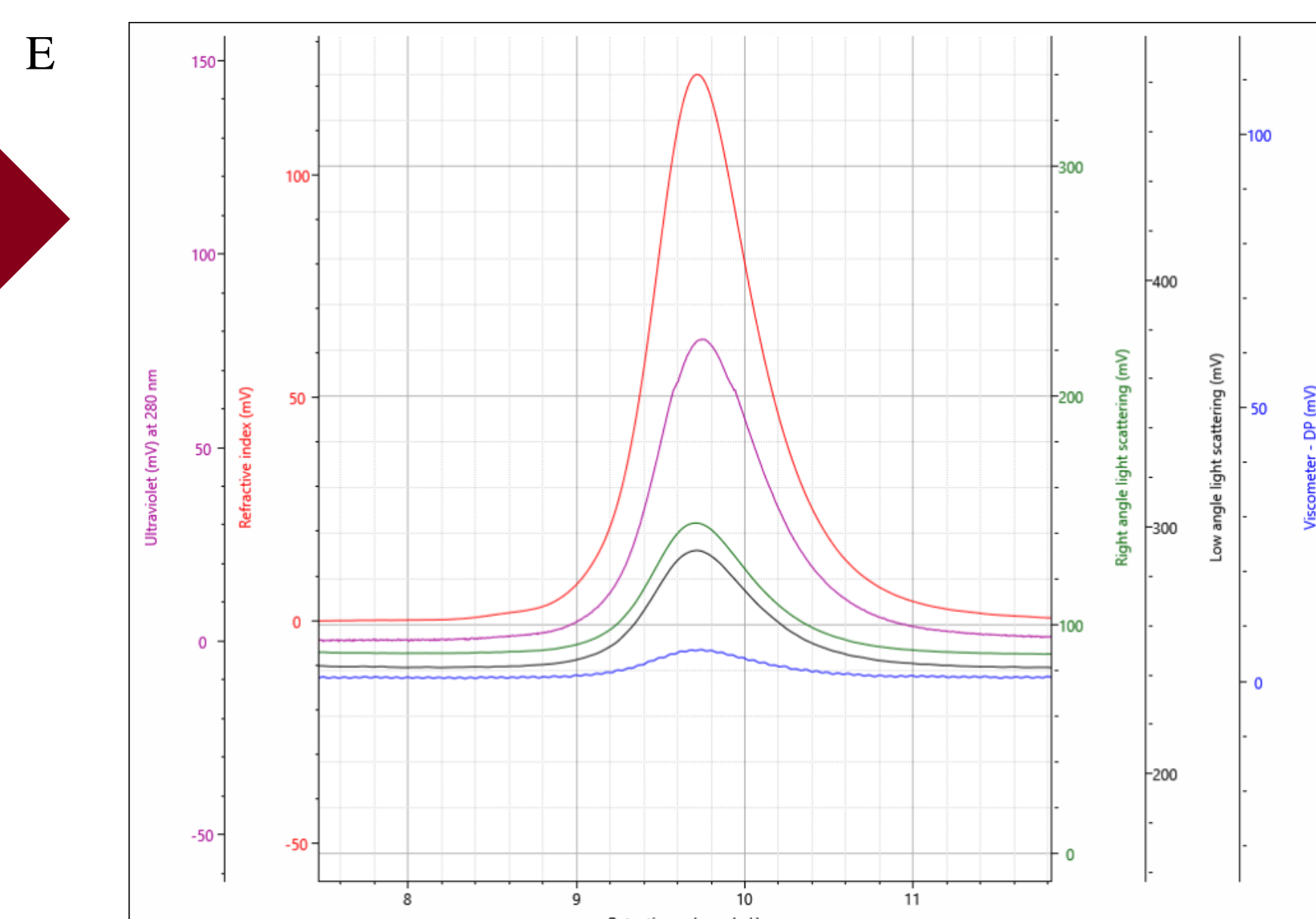


Table 2. Summary of the results of pure IgG from multi-detector SEC.

Parameter	Human IgG
V_R (mL)	9.71
M_w (Da)	154 215
M_w/M_n	1.002
IV (dL/g)	0.0586
R_h (nm)	5.2
Identity	Monomer

V_R - retention volume; M_w - molecular weight; M_w/M_n - polydispersity; IV - intrinsic viscosity; R_h - hydrodynamic radius

CONCLUSIONS

- The manufacturing procedure on a small-scale provided a high-quality IgG.
- CIM[®] monolithic anion-exchange chromatography proved sufficient for efficient removal of residual impurities, especially IgA and IgM as mediators of adverse transfusion reactions, enabling preparation of therapeutically relevant IgG-based product of improved safety.

ACKNOWLEDGMENT

The research has been financed by Croatian Science Foundation (grant IP-CORONA-04-2053) and by European Regional Development Fund, grant number KK.01.1.1.01.0006, "Strengthening the capacity of CerVirVac for research in virus immunology and vaccinology".

REFERENCES

- J. Abraham, *Nat. Rev. Immunol.* **2020**, *20*, 401-403.
- A. Casadevall, L. A. Pirofski, *J. Clin. Invest* **2020**, *130*, 1545-1548.
- S. Ali et al. *Immunotherapy* **2021**, *13*, 397-407.

Center of excellence
for
Virus Immunology and Vaccines

EUROPSKI STRUKTURNI
I INVESTICIJSKI FONDovi

EUROPSKA UNIJA
ZAJEDNO DO
FONDova EU

Operativni program
KONKURENTNOST
I KOHEZIJA