SUPPLEMENTARY MATERIAL TO

**Expression, purification and characterization of cellobiose dehydrogenase mutants from *Phanerochaete chrysosporium* in *Pichia pastoris* KM71H strain**

ANA MARIJA J. BALAŽ1, MARIJA B. BLAŽIĆ1, NIKOLINA POPOVIĆ2, OLIVERA L. PRODANOVIĆ3, RALUCA V. OSTAFE4, RAINER FISCHER5 and RADIVOJE M. PRODANOVIĆ2[[1]](#footnote-1)\*

*1Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoseva 12, 11000 Belgrade, Serbia*

*2Faculty of Chemistry, University of Belgrade, Studentski trg 12 – 16, 11000 Belgrade, Serbia*

*3* *Institute for Multidisciplinary Studies, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia*

*4* *Molecular Evolution Protein Engineering and Production facility (MEPEP), Purdue University, 207 S. Martin Jischke Dr., West Lafayette, IN 47907, USA*

*5Indiana Bioscience Research Institute, Single Cell Analytics Center, 1345 W. 16th St. Suite 300, Indianapolis, IN 46202, USA*

TABLE S1.Primers used for creation of triple mutant and error prone library mutants using wtCDH – pPICZαA vector as template

|  |  |
| --- | --- |
| Primer name | Primer sequence |
| Forward primer D20N | GGTATCACCAACCCTGTTCATG |
| Forward primer A64T | CTCGGTGGCACCATGAACAAC |
| Forward primer V592M | CGCAGCCTCCATGAACTCC |
| Forward primer D20N – V22A | CACCAACCCTGCTCATGACG |
| Forward primer T84A | TTTCCTCCGCTCGCTGG |
| Forward primer A261P | ACGTATGTCCCTCCATG |
| Forward primer E674G | AGACACTCGGGGAGTACG |
| Forward primer N715S | TTGGCACGAGCAACCTGTTT |

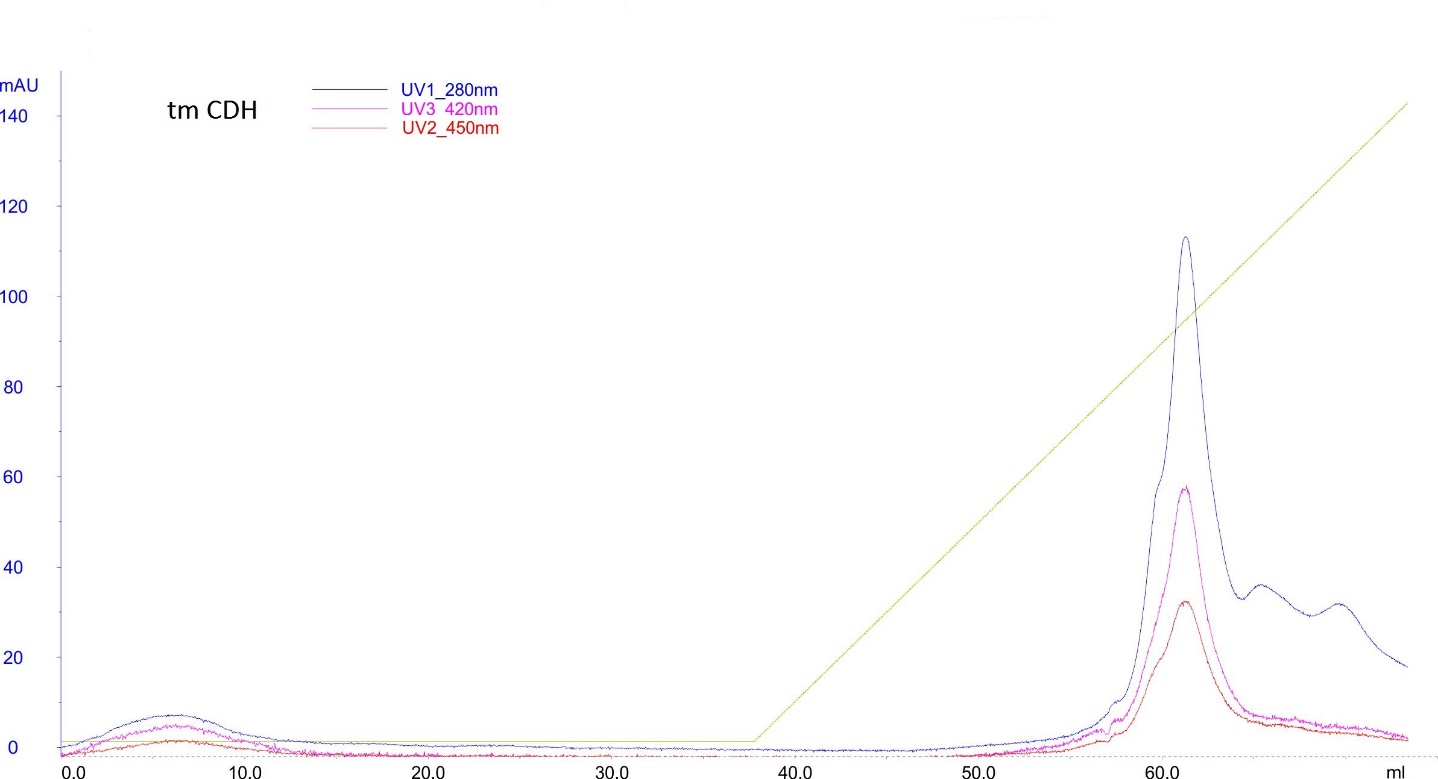


Fig. S1. Ion-exchange chromatography of tm CDH

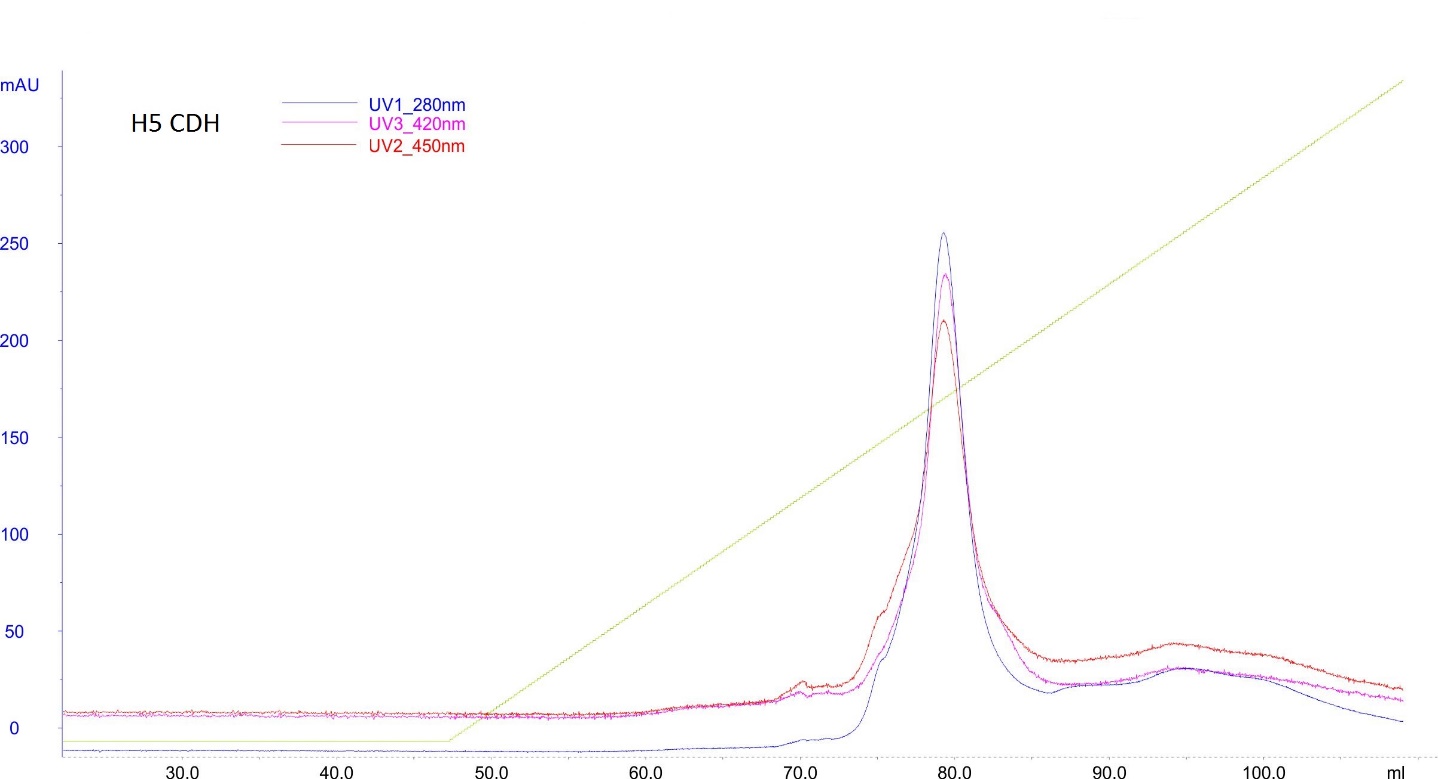


Fig. S2. Ion-exchange chromatography of H5 CDH

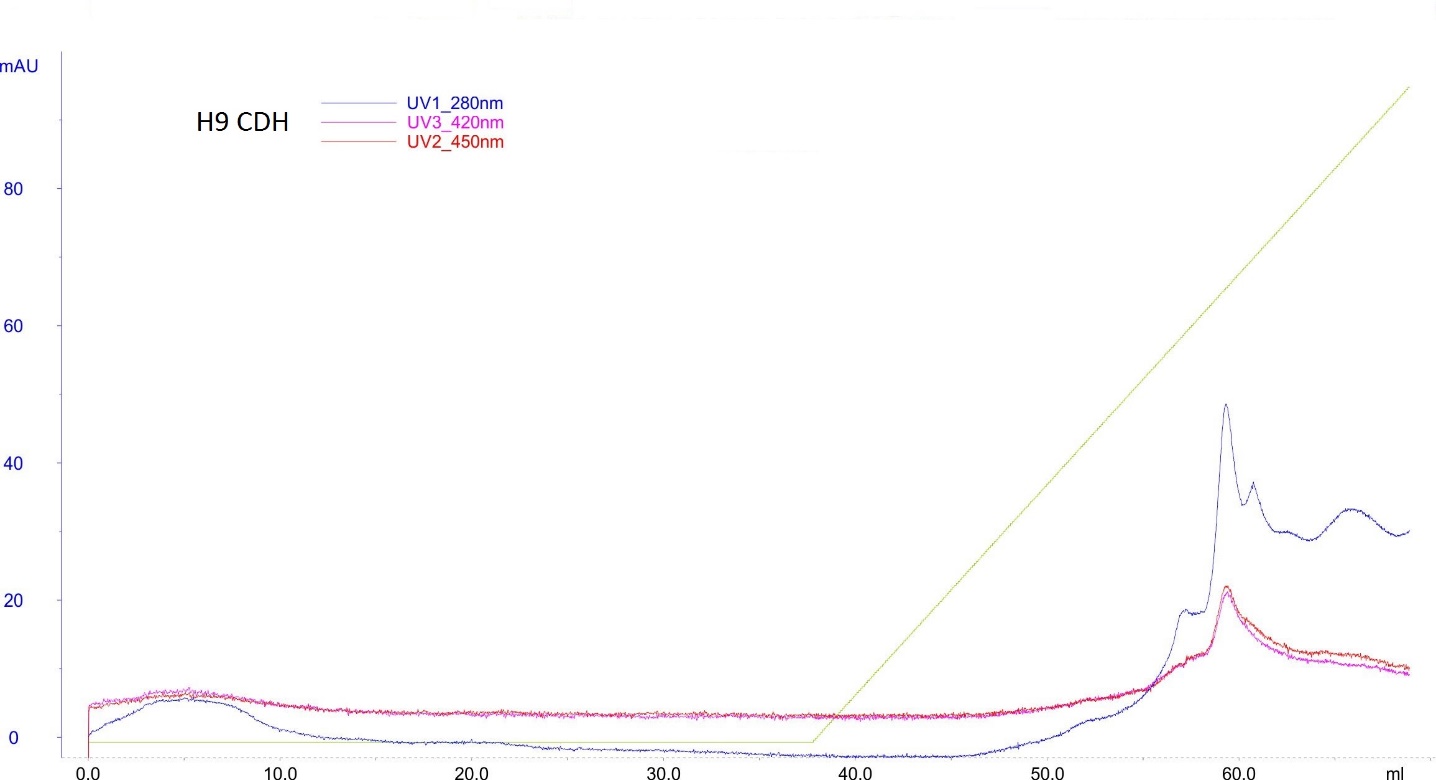


Fig. S3. Ion-exchange chromatography of H9 CDH

D:\NAUKA_Altos\BIOCHEMISTRY\Moji radovi\Revizija i priprema\AssocProf_20_AMB_2_JSCS\Revision 1\Figure S4.tif

Fig. S4. Ion-exchange chromatography of wt CDH

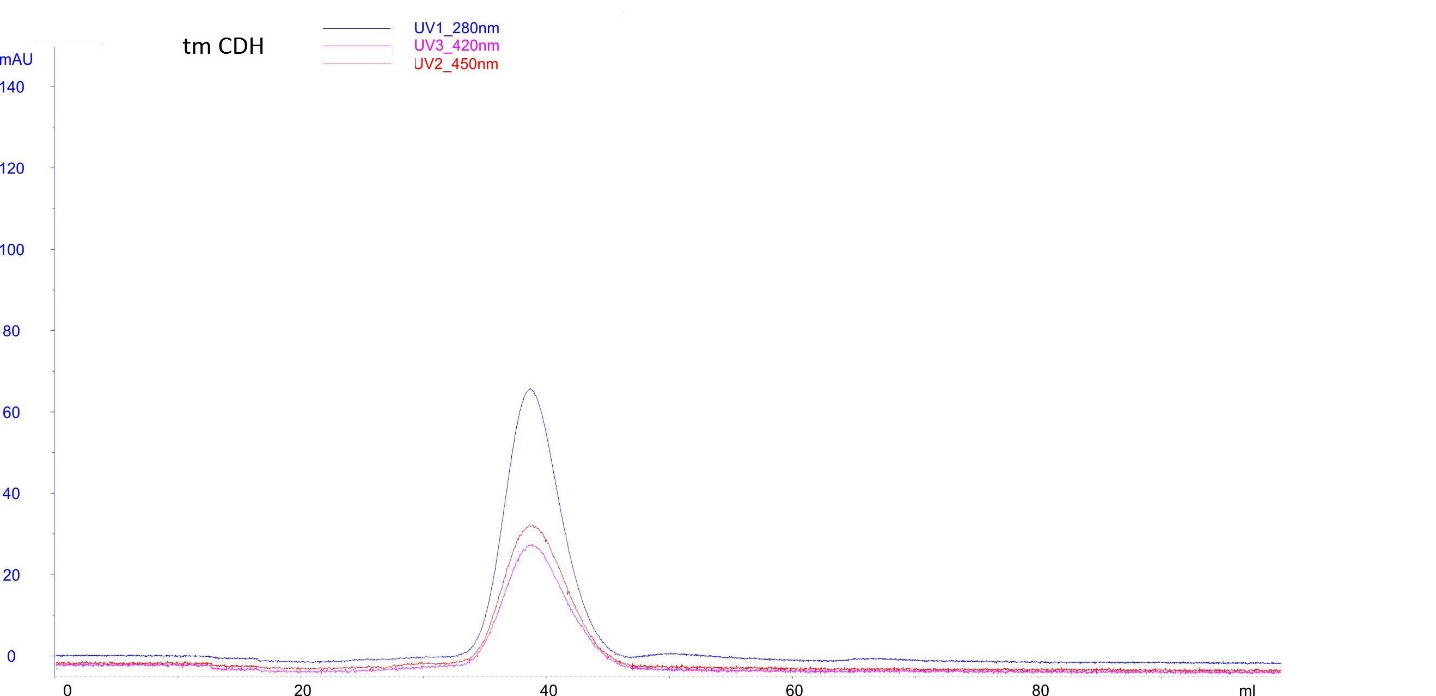


Fig. S5. Gel filtration of tm CDH

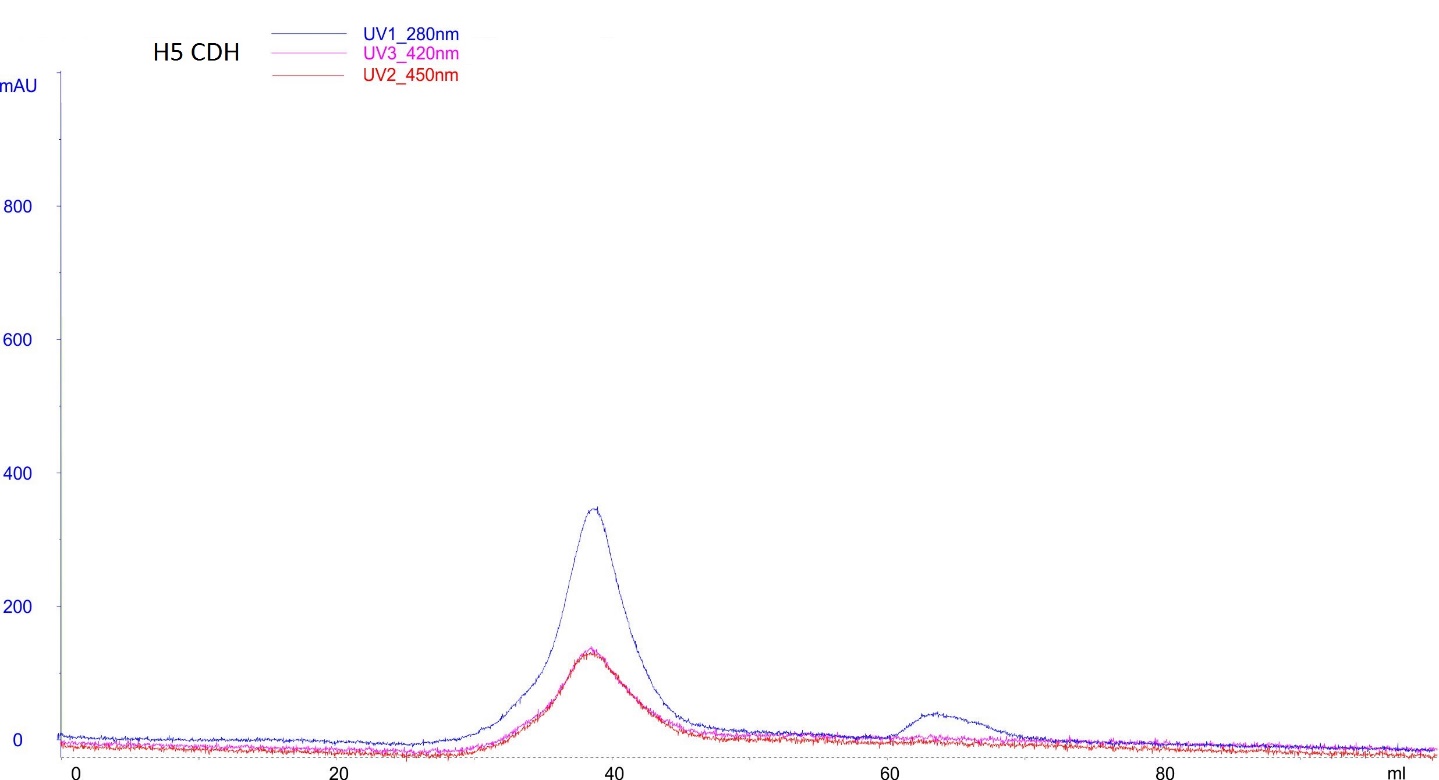


Fig. S6. Gel filtration of H5 CDH

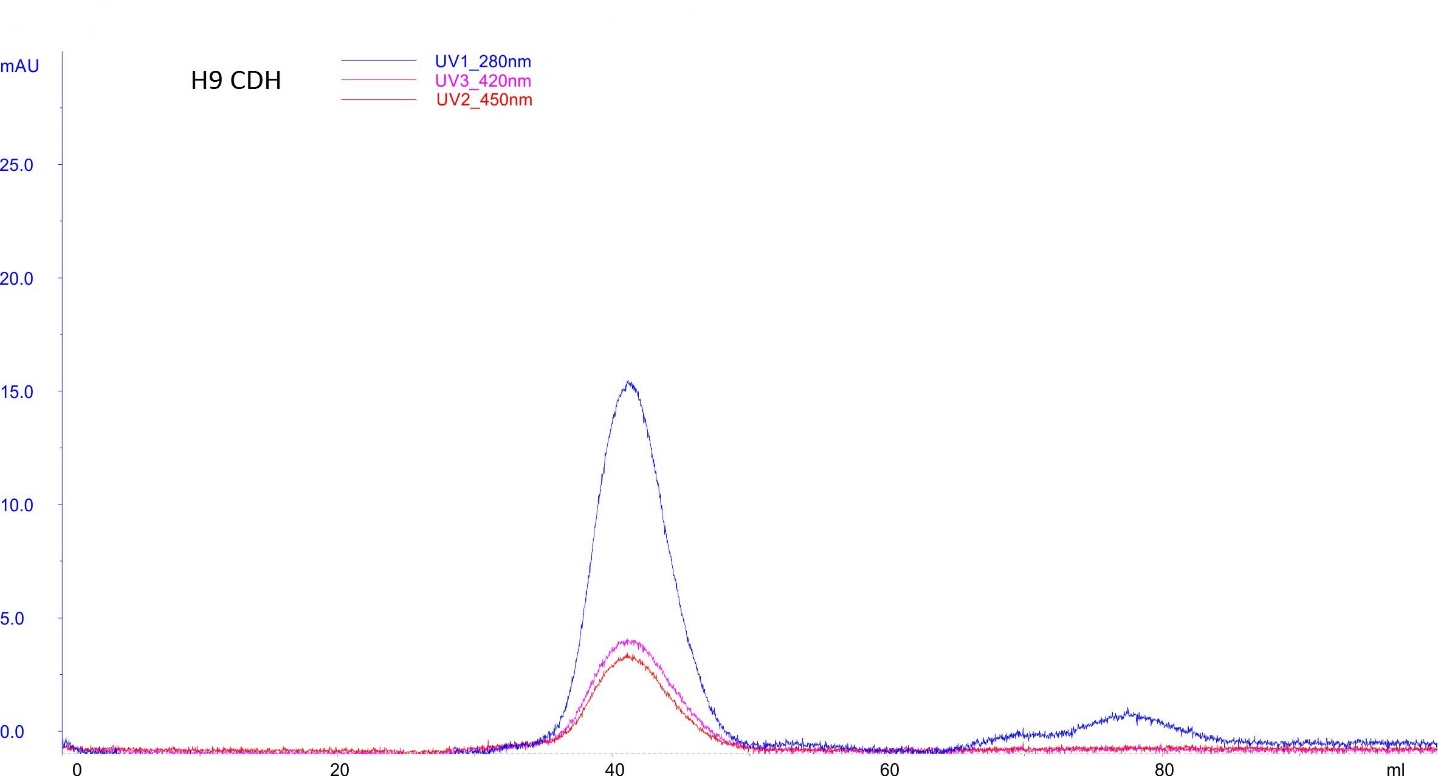


Fig. S7. Gel filtration of H9 CDH

D:\NAUKA_Altos\BIOCHEMISTRY\Moji radovi\Revizija i priprema\AssocProf_20_AMB_2_JSCS\Revision 1\Figure S8.tif

Fig. S8. Gel filtration of wt CDH

TABLE S2 Purification table for CDH.FM-fermentation media, UF-ultrafiltrate, DEAE-sample after ion-exchange chromatography, GF-sample after gel filtration. *Cp*-protein concentration, *Ac*-enzyme activity, *V*-volume, *Y*-yield of purification, *Pf*-purification factor, *Sp*-specific enzyme activity.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | tm CDH | | | | H5 CDH | | | | H9 CDH | | | | wt CDH | | | |
|  | FM | UF | DEAE | GF | FM | UF | DEAE | GF | FM | UF | DEAE | GF | FM | UF | DEAE | GF |
| *Cp* / mg mL-1 | 0.59 | 0.42 | 0.48 | 0.08 | 0.52 | 0.45 | 0.74 | 0.17 | 0.46 | 0.20 | 0.15 | 0.024 | 0.57 | 0.72 | 0.59 | 0.22 |
| *Ac* / IU mL-1 | 0.91 | 2.34 | 5.41 | 1.17 | 1.99 | 7.30 | 16.5 | 4.89 | 0.42 | 1.10 | 1.87 | 0.34 | 4.04 | 8.86 | 8.1 | 4.49 |
| *V* / mL | 50 | 12 | 3 | 7 | 50 | 12 | 3 | 6 | 50 | 12 | 3 | 5 | 50 | 12 | 5 | 6 |
| *Y* / % | 100 | 62 | 36 | 18 | 100 | 88 | 50 | 29 | 100 | 63 | 27 | 8.2 | 100 | 53 | 20 | 14 |
| *Pf* | 1 | 3.6 | 7.2 | 9.1 | 1 | 4.2 | 5.8 | 7.3 | 1 | 5.9 | 13 | 16 | 1 | 1.7 | 1.3 | 2.88 |
| *Sp* / IU mg-1 | 1.55 | 5.55 | 11.2 | 14.1 | 3.83 | 16.2 | 22.4 | 28.1 | 0.92 | 5.44 | 12.2 | 14.5 | 7.1 | 12.3 | 13.7 | 20.4 |

1. \*Corresponding author. E-mail: [rprodano@chem.bg.ac.rs](mailto:rprodano@chem.bg.ac.rs) [↑](#footnote-ref-1)