Supporting Information

Improving catalytic efficiency and stereoselectivity of a nitrilase from *Synechocystis sp.* PCC6803 by semi-rational engineering en route to chiral γ-amino acids

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Primers used in the mutation experiments

Table S1 Oligonucleotides used for mutagenesis

Primers	Sequence (5'→3')
P1	GGTCAT**CATATG**CTGAATTATACAAAAAATATTCG
Y59A	CAAAGGAAAAATAAGG**TGC**ATAAGGCACAAAAGTTTCAGG
F64A	CAAAACCGGCGGTTCAAC**TGC**GGAAAAATAAG
T139A	GTTCATGGTA**TGC**GGGCGTGATTTTG
Y140A	CGTTCATG**TGC**GGTGGGCGTGATTTTG
H141A	CCAAACCATCCGTTC**TGC**GTAGGTGG
E142A	CCAAACCATCCG**TGC**ATGGTAGGTGG
W170A	GGATTGTAATGTTC**TGC**ACAGGCCAAGGCTCC
F193A	CATCGATCCGGG**TGC**TTGCCCACAGTGG
P194A	CACCATCGATCCG**TGC**AATTGCCCACAG
M197A	GAAAATCTGACCCAC**TGC**CGATCCGGGGAATTG
V198A	CGAAAATCTGACC**TGC**CATCGATCCGG
I201A	CGAAAATCTGACC**TGC**CATCGATCCGG
F202A	CCATTTGATCCGC**TGC**AATCTGACCCACC
Q205A	GATCCGCGAA**TGC**CTGACCCACCATC
Saturation mutagenesis sites	
H141	CCAAACCATCCGTTC**AHN**GTAGGTGGGCGTGATTTTGC
P194	GACCCACCATCGATCC**AHN**GAATTGCCCACAGTGG
M197	GAAAATCTGACCCAC**AHN**CGATCCGGGGAATTGC
I201	CATTTGATCCGCGAA**AHN**CTGACCCACCATCG
F202	CCATTTGATCCGC**AHN**AATCTGACCCACC

Alanine scanning

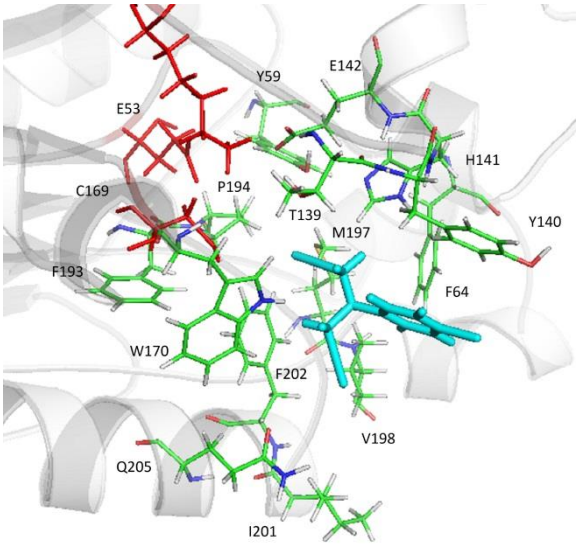


Figure S1 Modeling 1a (cyan) into the active site of the wt *Ss*NIT by Discovery Studio 4.1 that highlights fourteen amino acid residues with carbon atoms in green located within a distance of 5 Å of 1a as targets for alaine scanning. The catalytic residues, E53, K135, and C169, are represented by red.

The reaction of alanine scanning,

To a 1 mL reaction, **1a** (10 mM) and whole cells (OD_{600}=5) were added to phosphoric acid (100 mM, pH 7.0). The reaction mixture was incubated at 30°C with 200 rpm for 30 min, and then quenched with 20 µL of 6 M HCl solution. 1 mL of ethyl acetate was added to the mixture and organic phases were dried with anhydrous sodium sulfate for HPLC analysis.

Protein expression of WT and mutants

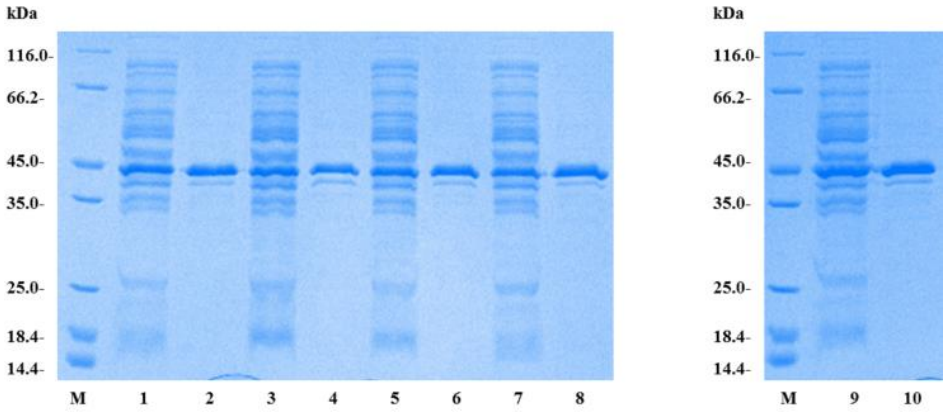


Figure S2 SDS-PAGE analysis of the recombinant nitrilase. M: protein standard marker; Lane 1: supernatant of wt *Ss*NIT; Lane 2: precipitate of wt *Ss*NIT; Lane 3: supernatant of P194A; Lane 4: precipitate of P194A; Lane 5: supernatant of F202V; Lane 6: precipitate of F202V; Lane 7: supernatant of P194A/F202V; Lane 8: precipitate of P194A/F202V; Lane 9: supernatant of P194A/I201A/F202V; Lane 10: precipitate of P194A/I201A/F202V;

Chiral HPLC analysis, ^{1}H-NMR and ^{13}C-NMR of product ((*S*)-2a) catalyzed by whole cells of variant P194A/I201A/F202V

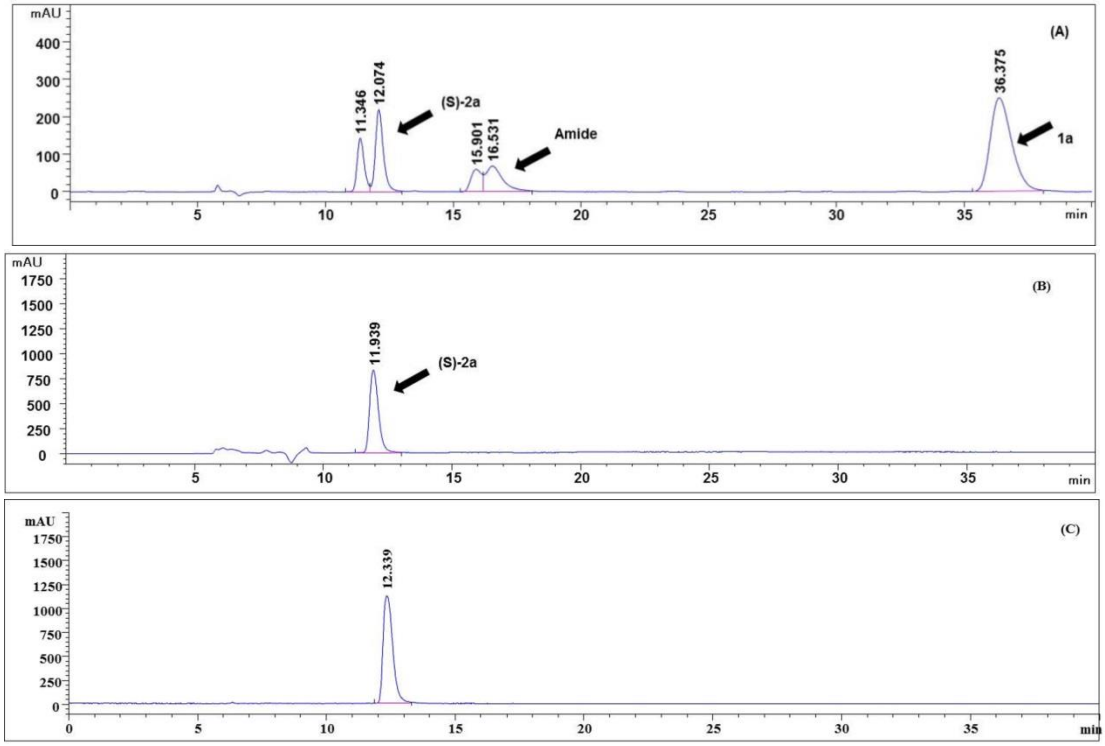


Figure S3. HPLC traces of the mixture of the standards of rac-3-(4-chlorophenyl)-4-cyanobutanoic acid (**2a**), rac-amide and 3-(4-chlorophenyl) glutaronitrile (**1a**) (A), the product ((*S*)-**2a**) catalyzed by whole cells of *Bj*NIT6402 from *Bradyrhizobium japonicum* USDA110 (B), and product ((*S*)-**2a**) catalyzed by whole cells of variant P194A/I201A/F202V (C).

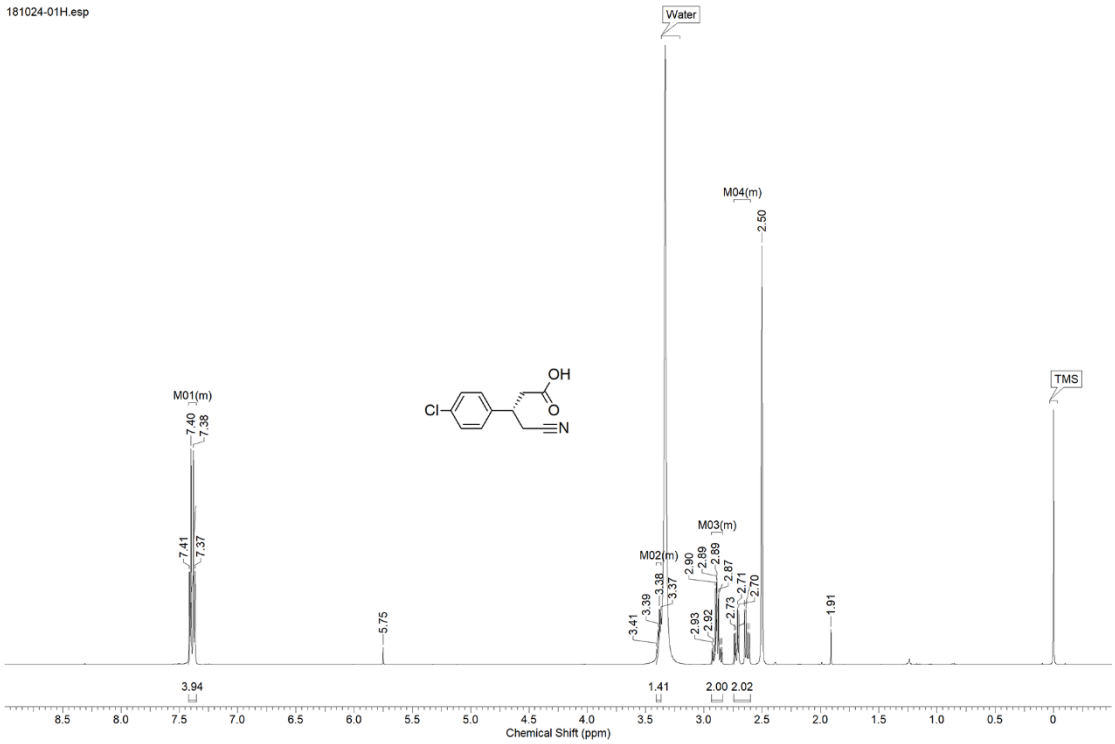


Figure S4 ^{1}H-NMR of product ((*S*)-**2a**) catalyzed by whole cells of variant P194A/I201A/F202V.

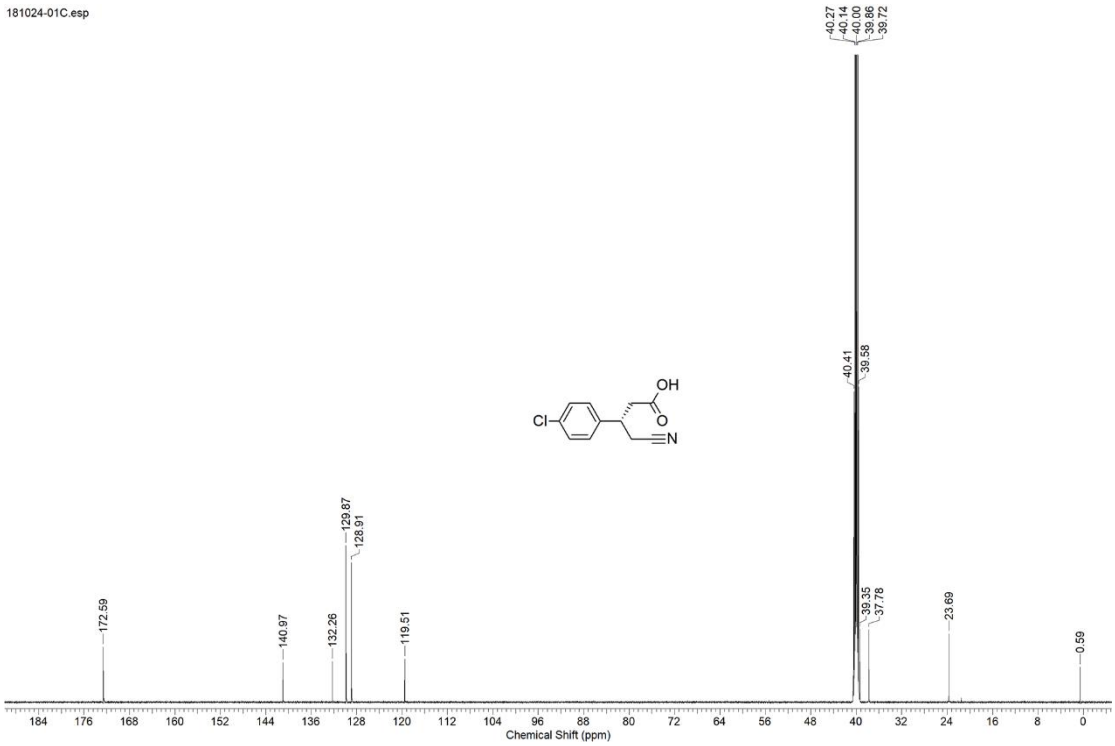


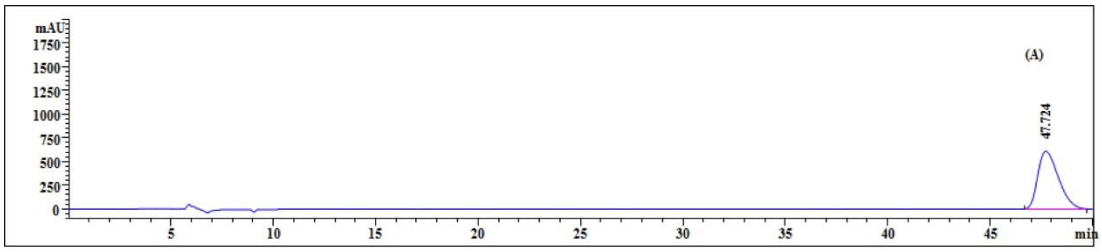
Figure S5 ^{13}C-NMR of product ((*S*)-**2a**) catalyzed by whole cells of variant P194A/I201A/F202V.

Analytical reaction of other 3-substituted glutaronitriles 1b-n catalyzed by whole cells of wt *Ss*NIT and its mutant P194A/I201A/F202V

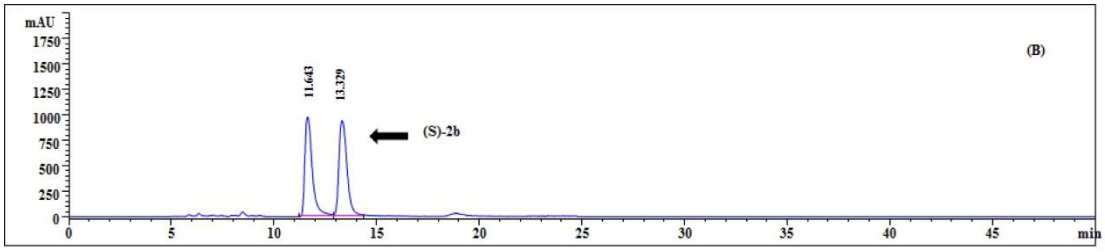
All the samples were detected using HPLC with a UV detector at the wavelength of 210 nm by a CHIRALPAK OD-H column (5 μm, 4.6 mm × 250 mm) and isocratically eluted at 30°C. The flow rate was 0.5 mL/min. The eluent of isopropanol-hexane-trifluoroacetic acid was 30:70:0.1 (v/v/v), except that 1f and 2f were 10:90:0.1(v/v/v) for 30 min and then 40:60:0.1(v/v/v) for 50 min .

(1) The reaction of **1b**

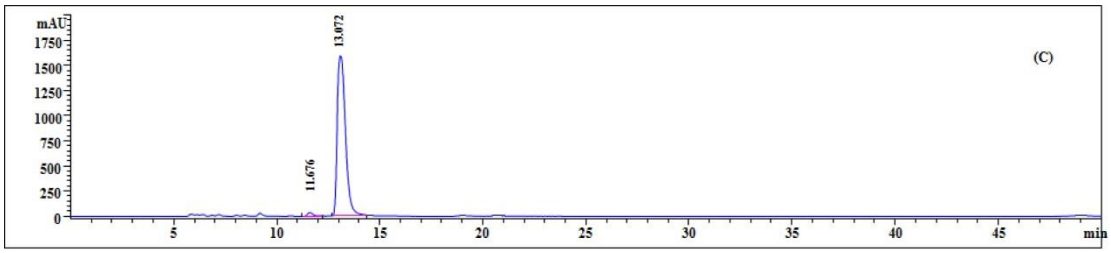
Standard sample of **1b**



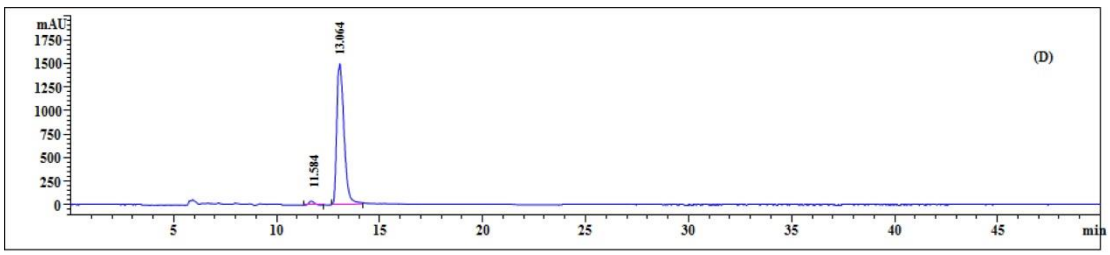
Racemate of **2b**



Sample of **2b** with wt *Ss*NIT

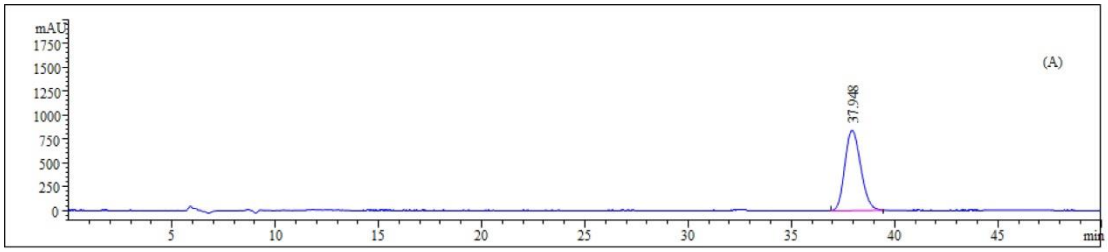


Sample of **2b** with P194A/I201A/F202V

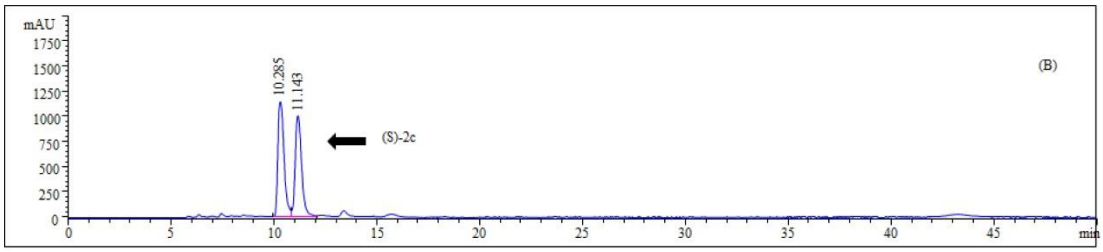


(2) The reaction of **1c**

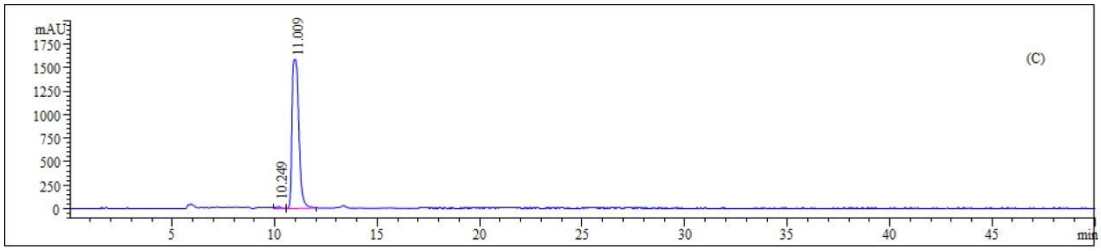
Standard sample of **1c**



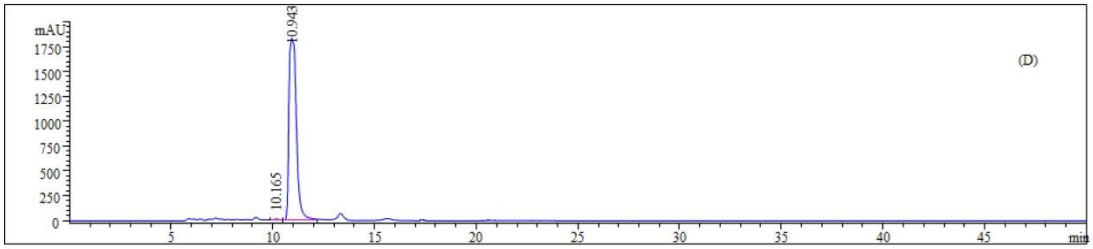
Racemate of **2c**



Sample of **2c** with wt *Ss*NIT

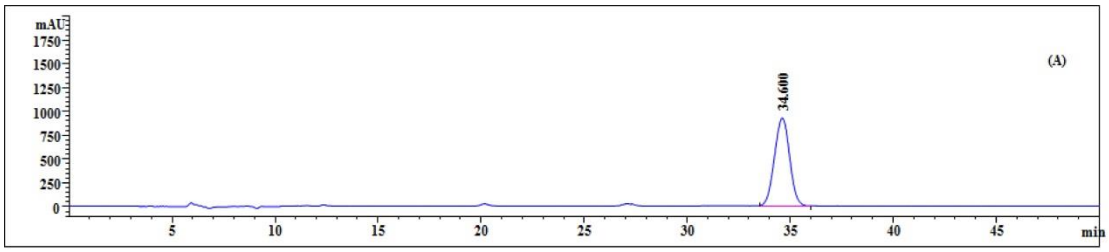


Sample of **2c** with P194A/I201A/F202V

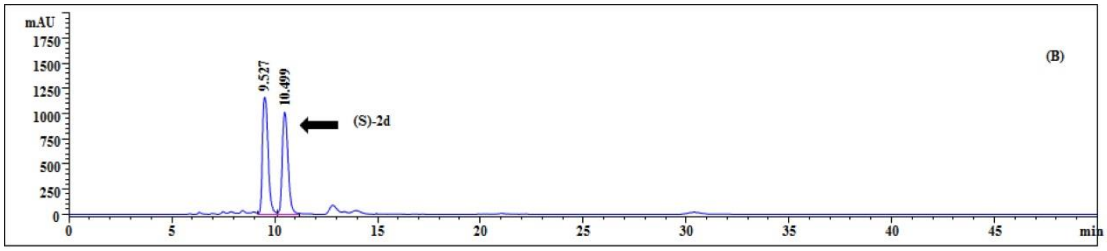


(3) The reaction of **1d**

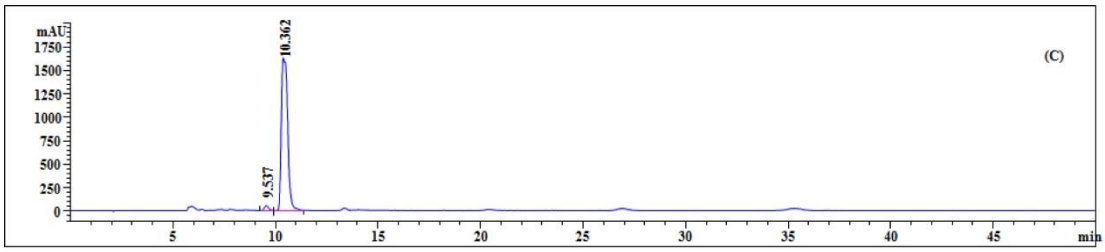
Standard sample of **1d**



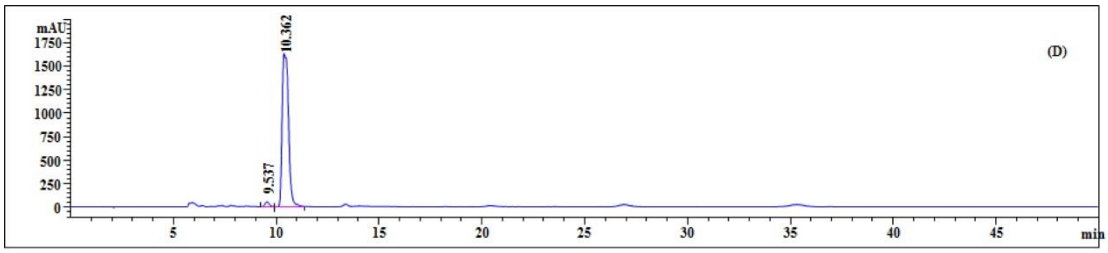
Racemate of **2d**



Sample of **2d** with wt *Ss*NIT

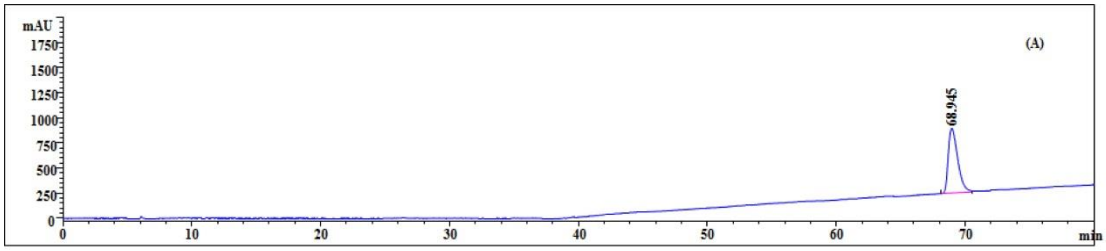


Sample of **2d** with P194A/I201A/F202V

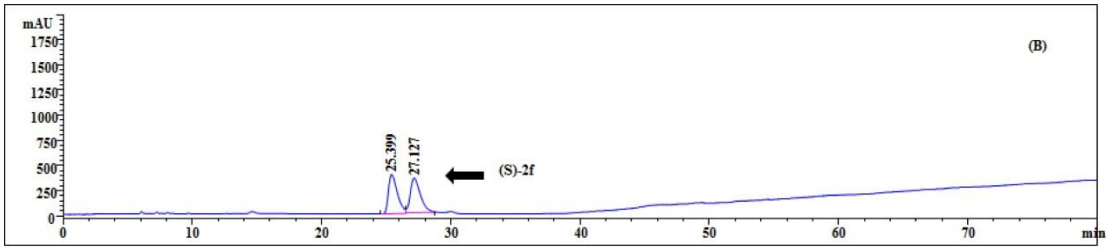


(4) The reaction of **1f**

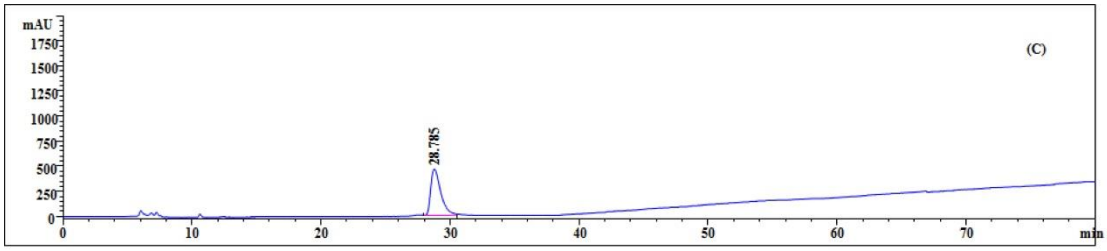
Standard sample of **1f**



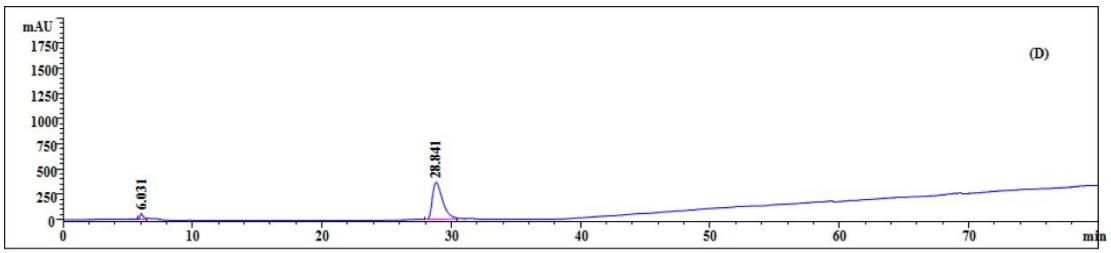
Racemate of **2f**



Sample of **2f** with wt *Ss*NIT

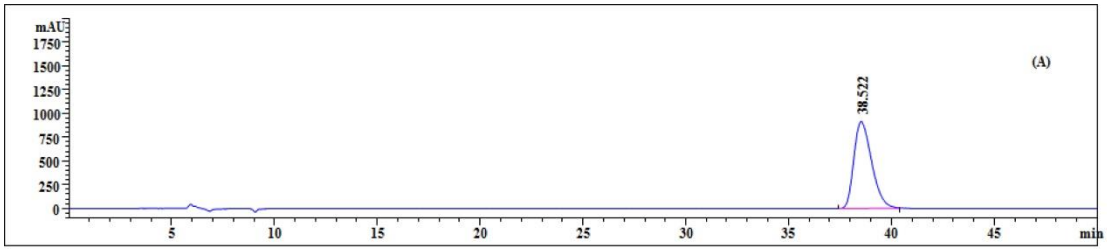


Sample of **2f** with P194A/I201A/F202V

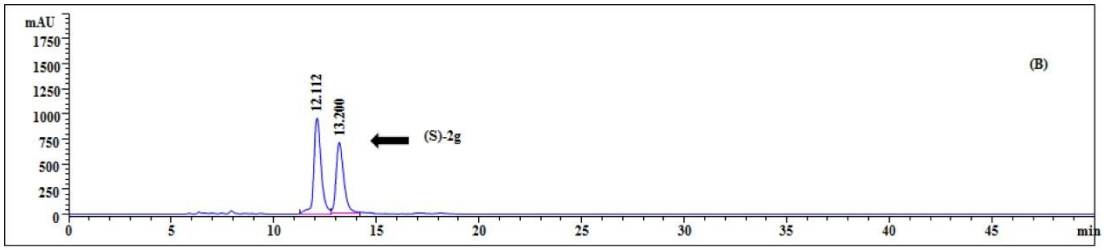


(5) The reaction of **1g**

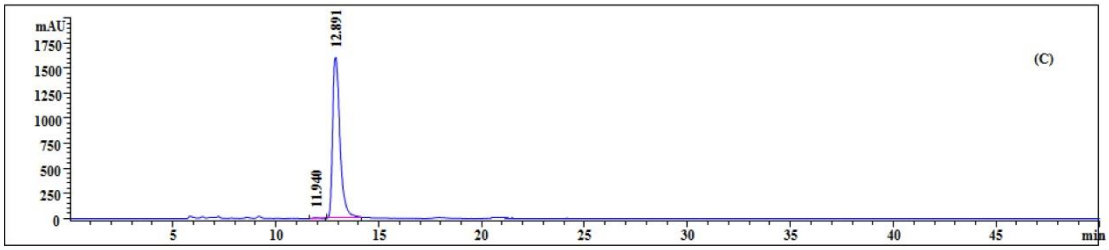
Standard sample of **1g**



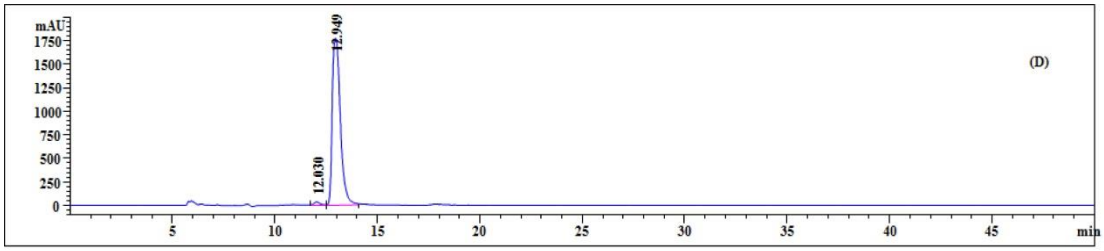
Racemate of **2g**



Sample of **2g** with wt *Ss*NIT

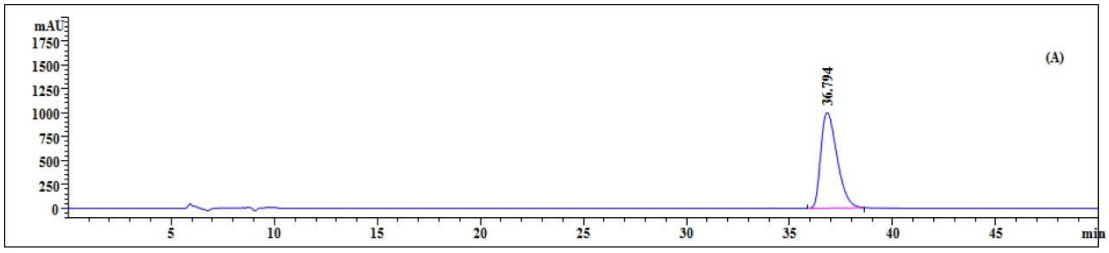


Sample of **2g** with P194A/I201A/F202V

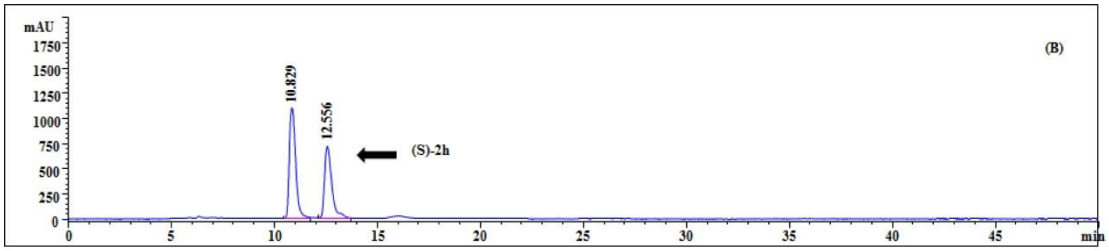


(6) The reaction of **1h**

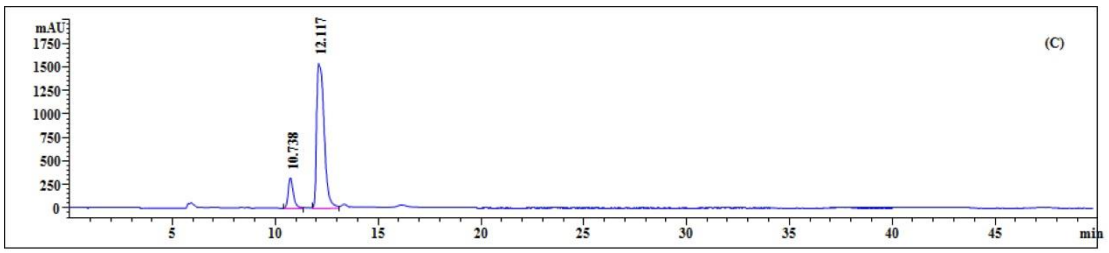
Standard sample of **1h**



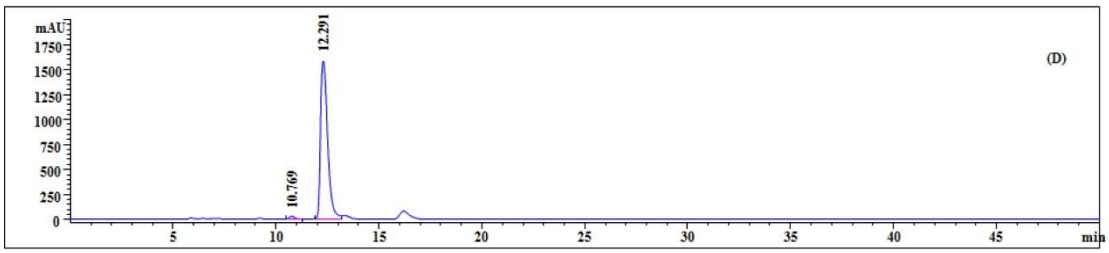
Racemate of **2h**



Sample of **2h** with wt *Ss*NIT

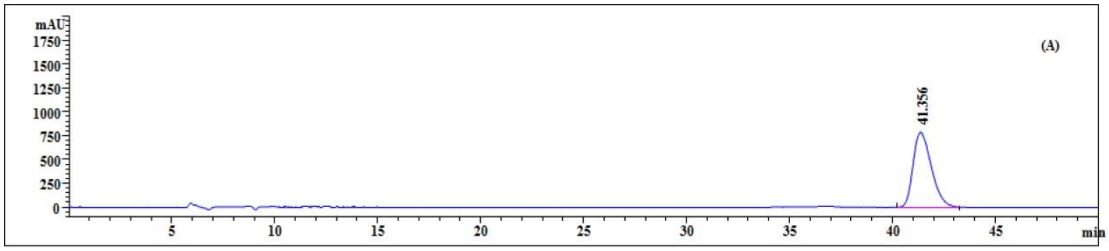


Sample of **2h** with P194A/I201A/F202V

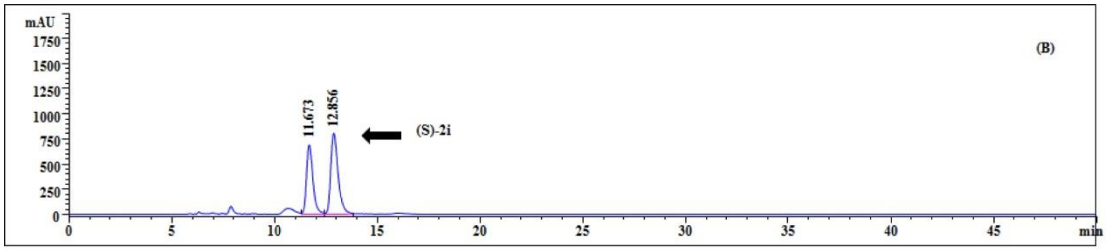


(7) The reaction of **1i**

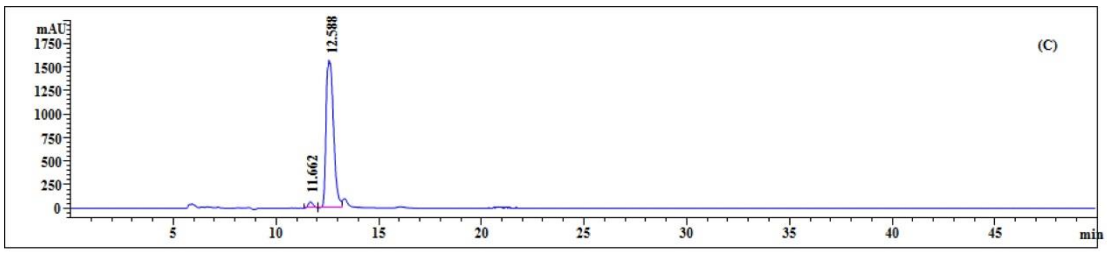
Standard sample of **1i**



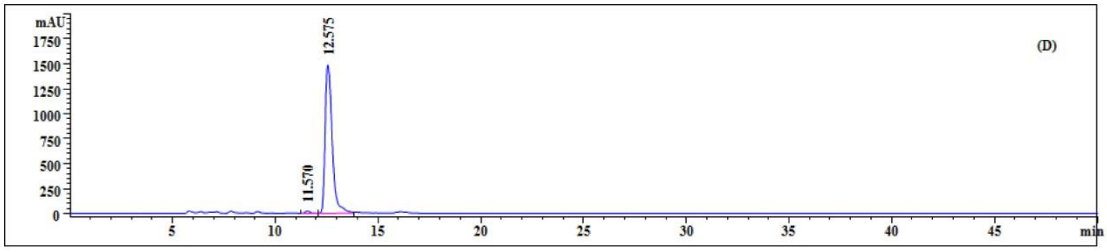
Racemate of **2i**



Sample of **2i** with wt *Ss*NIT

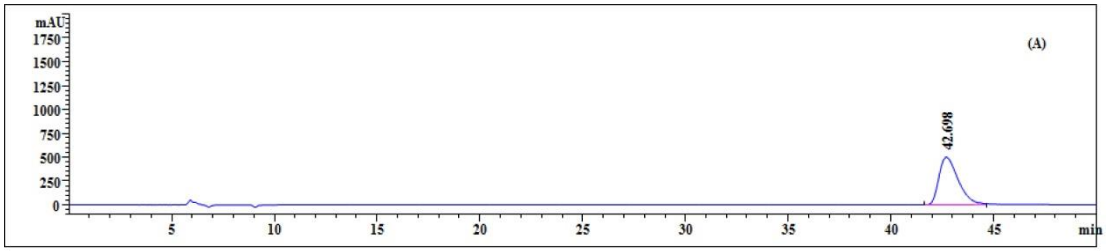


Sample of **2i** with P194A/I201A/F202V

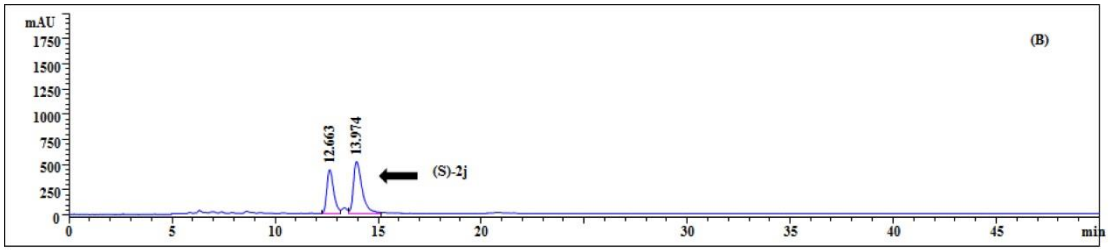


(8) the reaction of **1j**

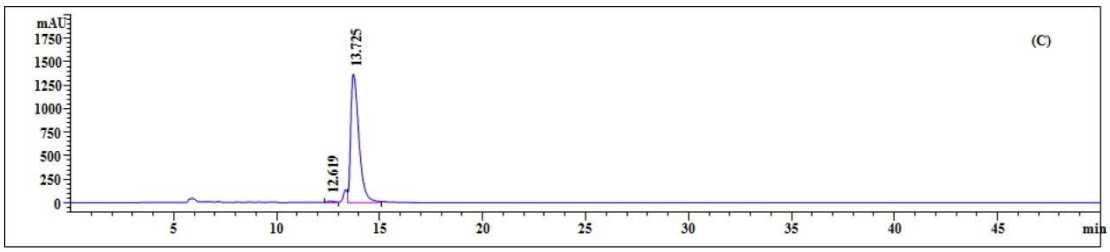
Standard sample of **1j**



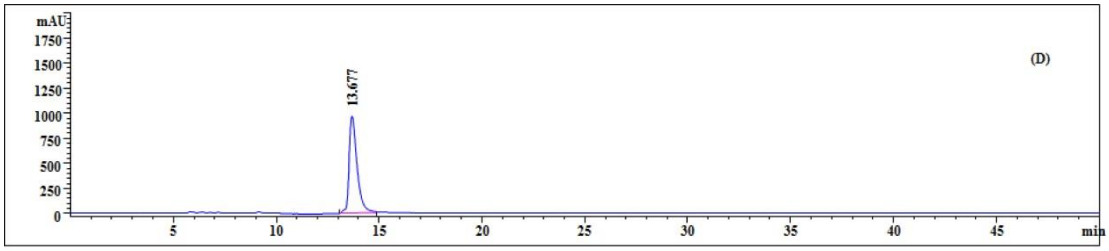
Racemate of **2j**



Sample of **2j** with wt *Ss*NIT

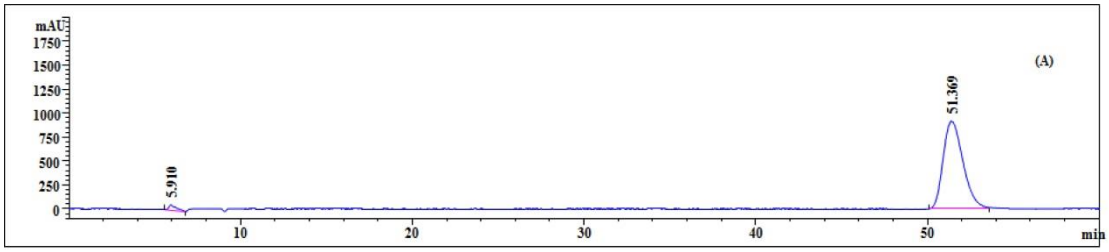


Sample of **2j** with P194A/I201A/F202V

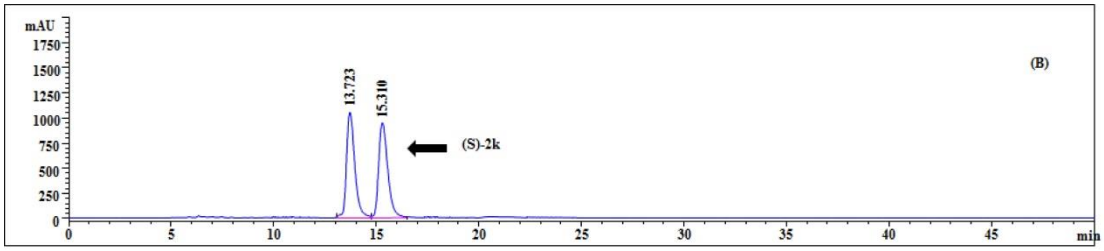


(9) The reaction of **1k**

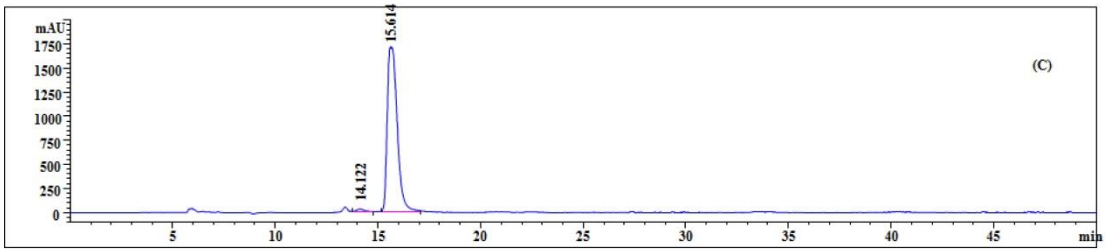
Standard sample of **1k**



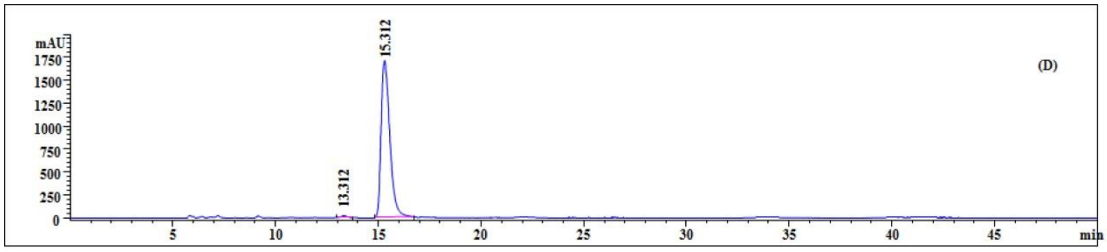
Racemate of **2k**



Sample of **2k** with wt *Ss*NIT

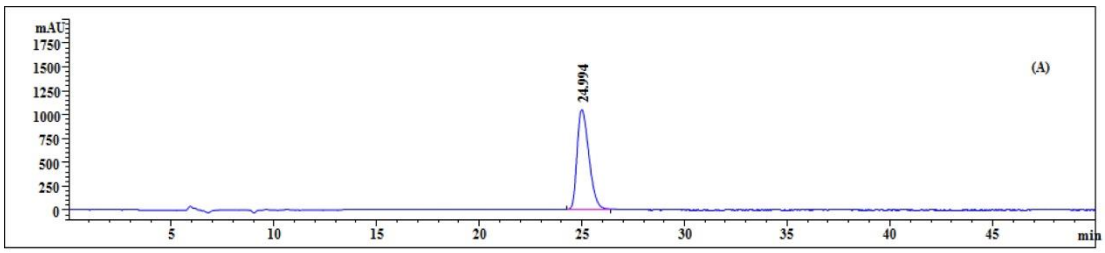


Sample of **2k** with P194A/I201A/F202V

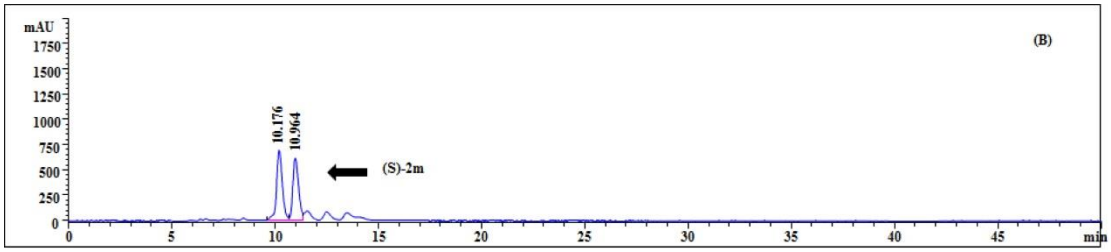


(10) The reaction of **1m**

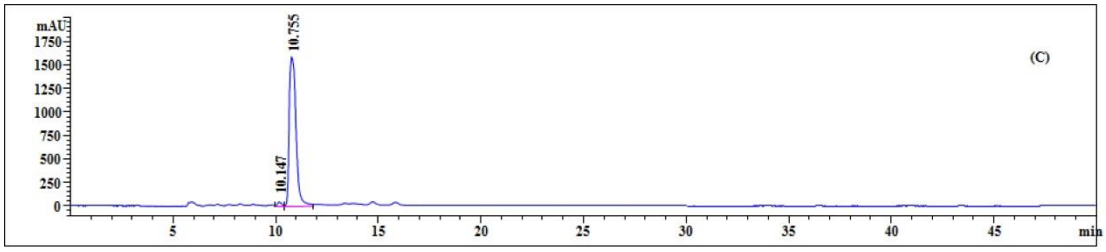
Standard sample of **1m**



Racemate of **2m**



Sample of **2m** with wt *Ss*NIT



Sample of **2m** with P194A/I201A/F202V