Supporting Information

Efficient Kinetic Resolution of Racemic Amines using a Transaminase in Combination with an Amino Acid Oxidase

Matthew D. Truppo*, Nicholas J. Turner* and J. David Rozzell

School of Chemistry, University of Manchester, Manchester Interdisciplinary Biocentre, 131 Princess Street, Manchester M1 7DN, UK

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General Experimental:

Commercial grade reagents and solvents were purchased from Sigma-Aldrich and used without further purification. All enzymes including transaminases (ATAs), amino acid oxidase (AAO), glucose dehydrogenase (GDH), and lactate dehydrogenase (LDH) were generously supplied by Codexis (Redwood City, CA).

HPLC Assay Conditions

Reaction conversion was monitored using reverse phase high performance liquid chromatography (HPLC) at 210 nm using an Agilent 1100 series HPLC and a Zorbax Eclipse XDB-C18 (50 x 4.6 mm) column with a flow rate of 1 mL/min (60% acetonitrile / 40% water).

Enantiomeric excess was determined by normal phase high performance liquid chromatography (HPLC) at 210 nm using an Agilent 1100 series HPLC and a Chiralpak OD-H (250 x 4.6 mm) column with a flow rate of 1 mL/min (70% hexanes / 30% ethanol). Specific rotation of the amine product was established by comparison to known standards purchased from Sigma-Aldrich.

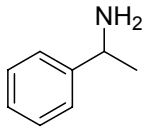
General Amine Resolution Conditions:

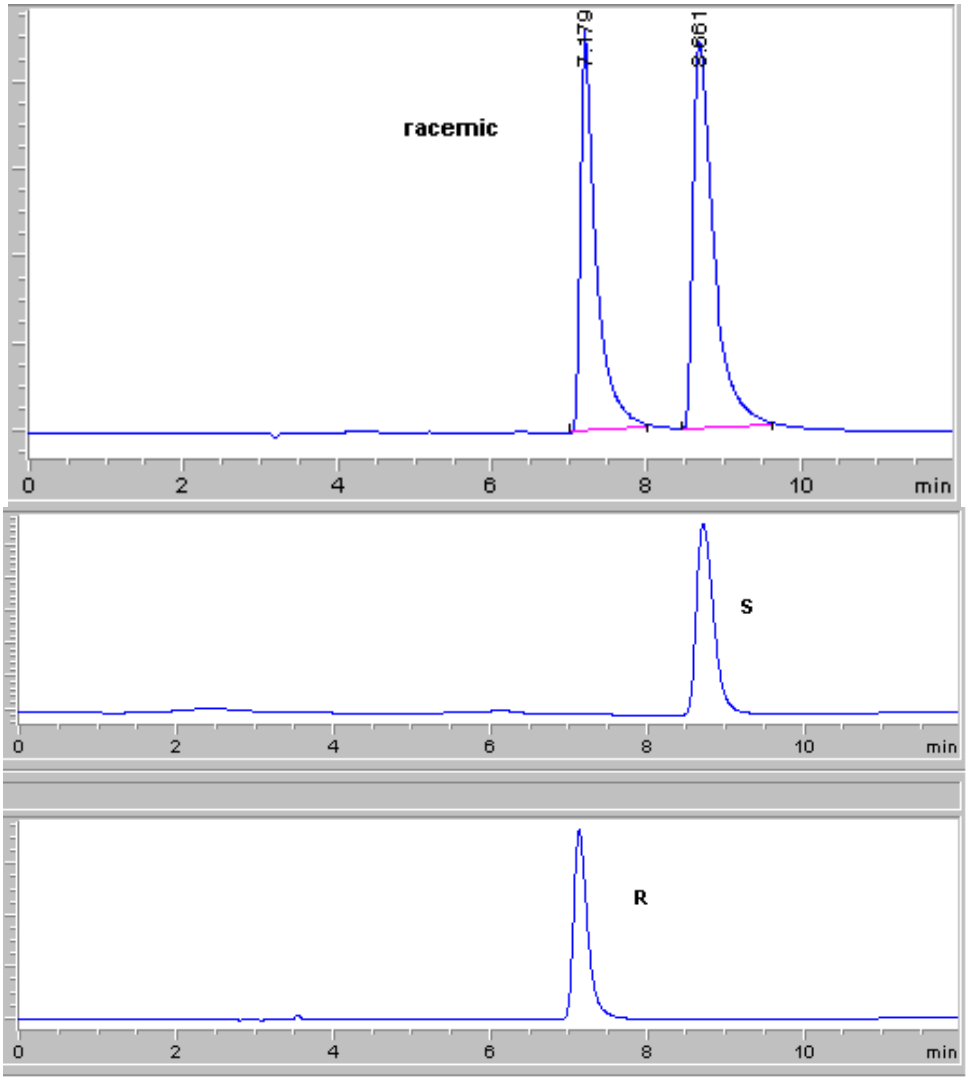
The resolution of various amines was run in 100 mM potassium phosphate buffer using the following conditions and concentrations: 30 ºC, pH 8.0, 1 g/L transaminase (ATA) enzyme, 1 g/L amino acid oxidase (AAO), 0.2 g/L pyridoxal-5-phosphate cofactor, 2 mM pyruvate, and 25 mM racemic amine substrate. Reactions were run for 3 hours and then assayed by HPLC. Samples for reverse phase HPLC were diluted 1:10 with acetonitrile, filtered and run using the method described above. Samples for normal phase HPLC were extracted with methyl tertbutyl ether (MTBE), dried down, re-suspended in the mobile phase (70% hexanes / 30% ethanol), and run according to the method described above.

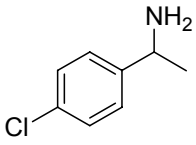
Optimized Methylbenzylamine Resolution Conditions:

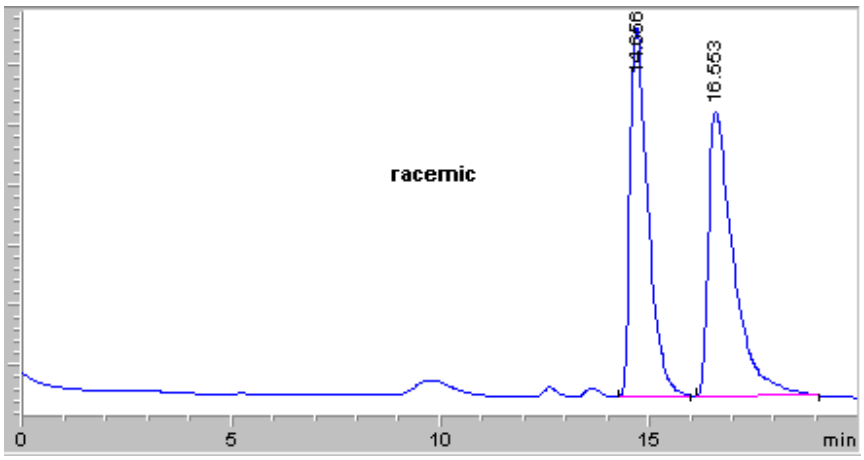
The resolution of methylbenzylamine was run in 100 mM potassium phosphate buffer using the following conditions and concentrations: 30 ºC, pH 8.0, 1 g/L transaminase (ATA) enzyme, 1 g/L amino acid oxidase (AAO), 0.2 g/L pyridoxal-5-phosphate cofactor, 0.1 mM pyruvate, and 100 mM methylbenzylamine. Reaction samples were taken over a 12 hour time course until 50% conversion was observed. Samples for reverse phase HPLC were diluted 1:10 with acetonitrile, filtered and run using the method described above. Samples for normal phase HPLC were extracted with methyl tertbutyl ether (MTBE), dried down, re-suspended in the mobile phase (70% hexanes / 30% ethanol), and run according to the method described above.

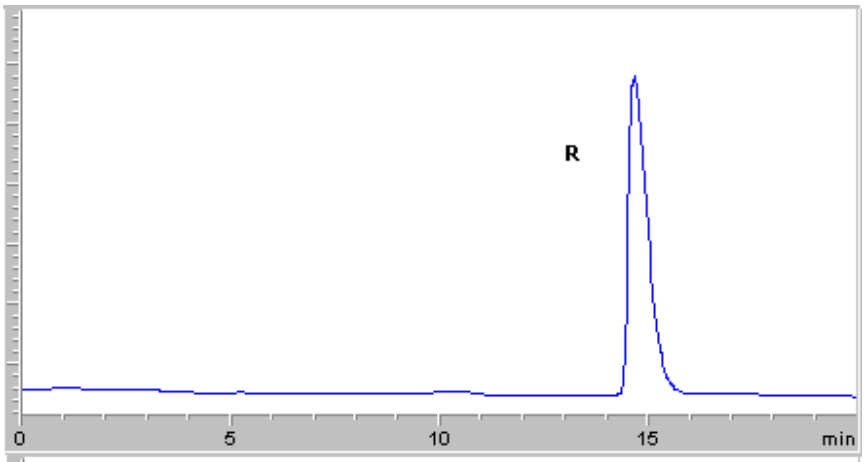
Amine Normal Phase Chiral HPLC Chromatograms:

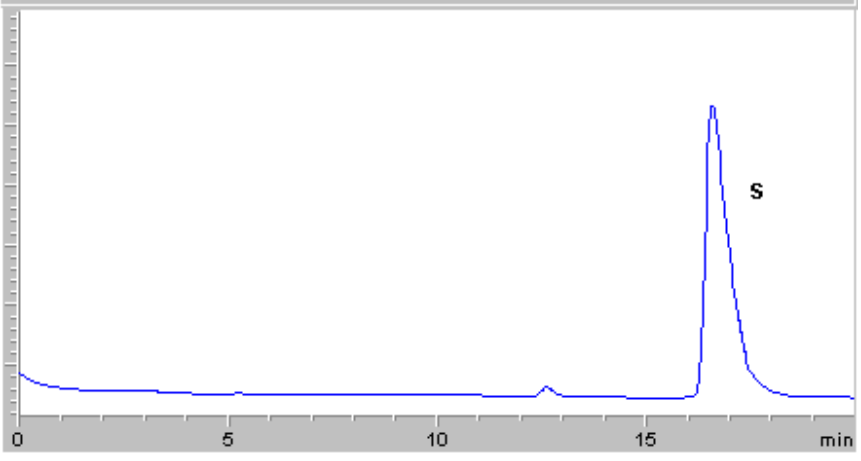


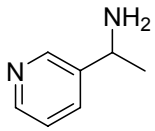


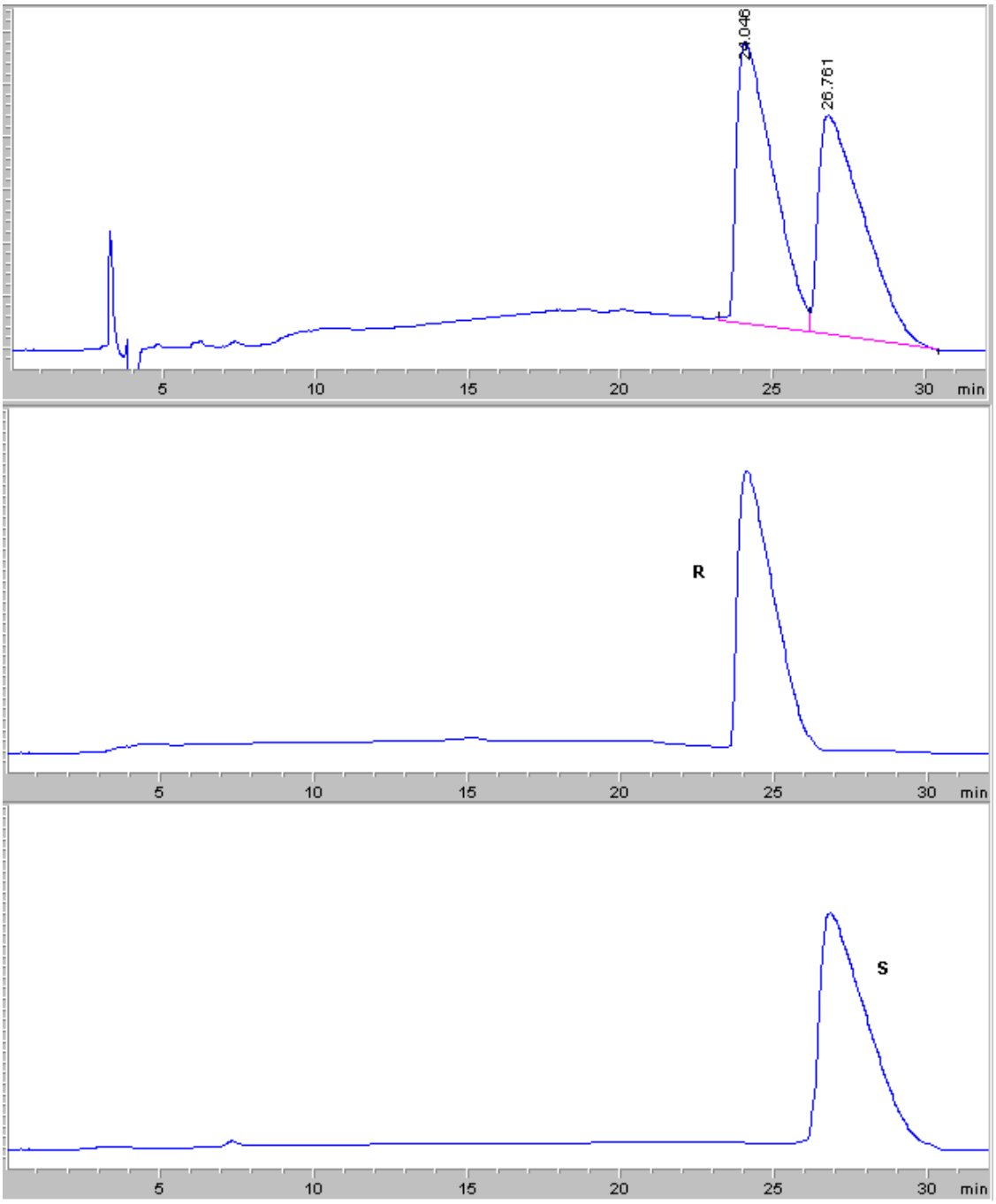

NH2
Cl

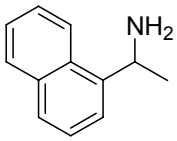

racemic
16.553
0
5
10
15
min

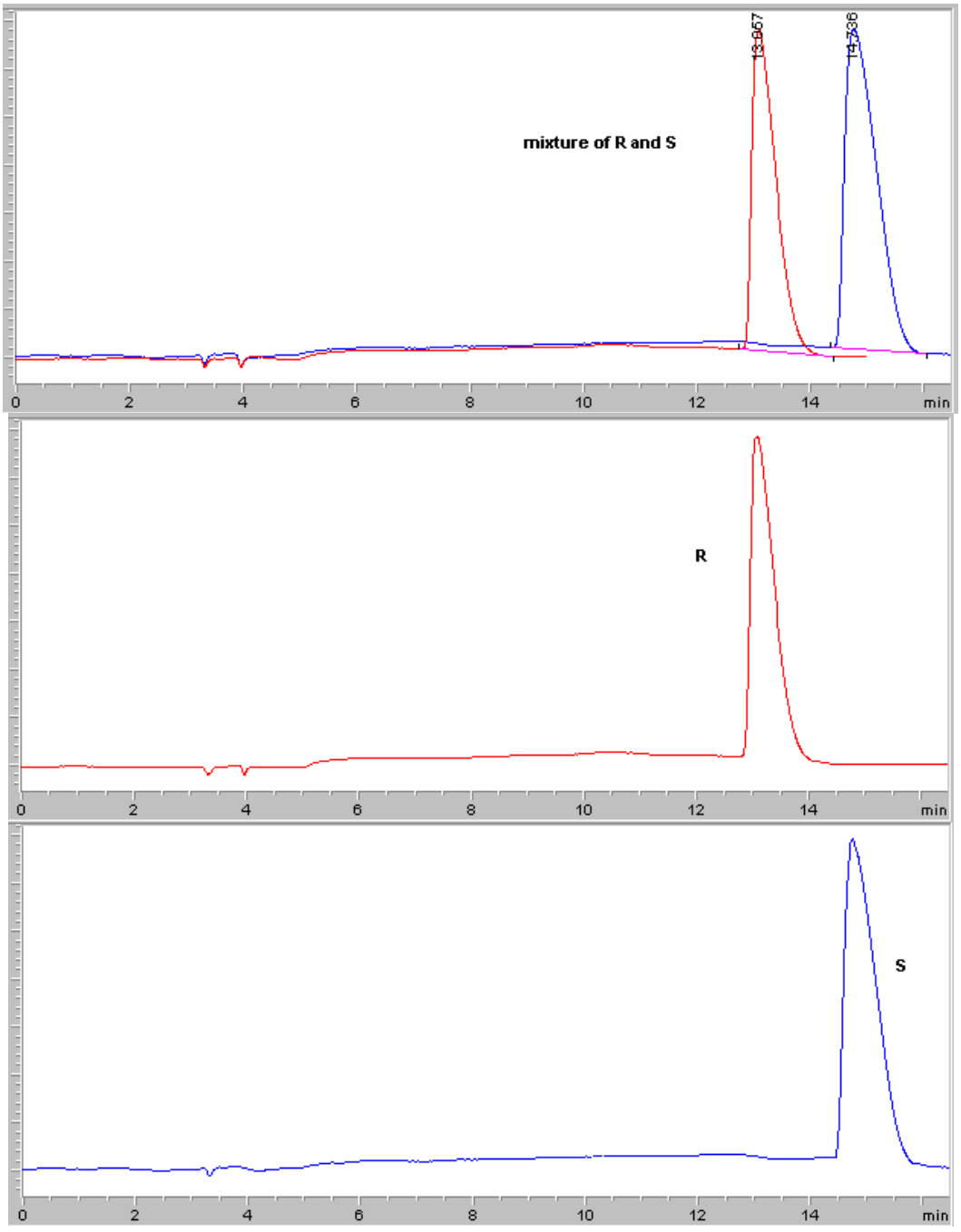

R
0
5
10
15
min

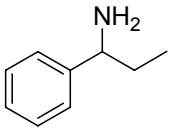

S
0
5
10
15
min

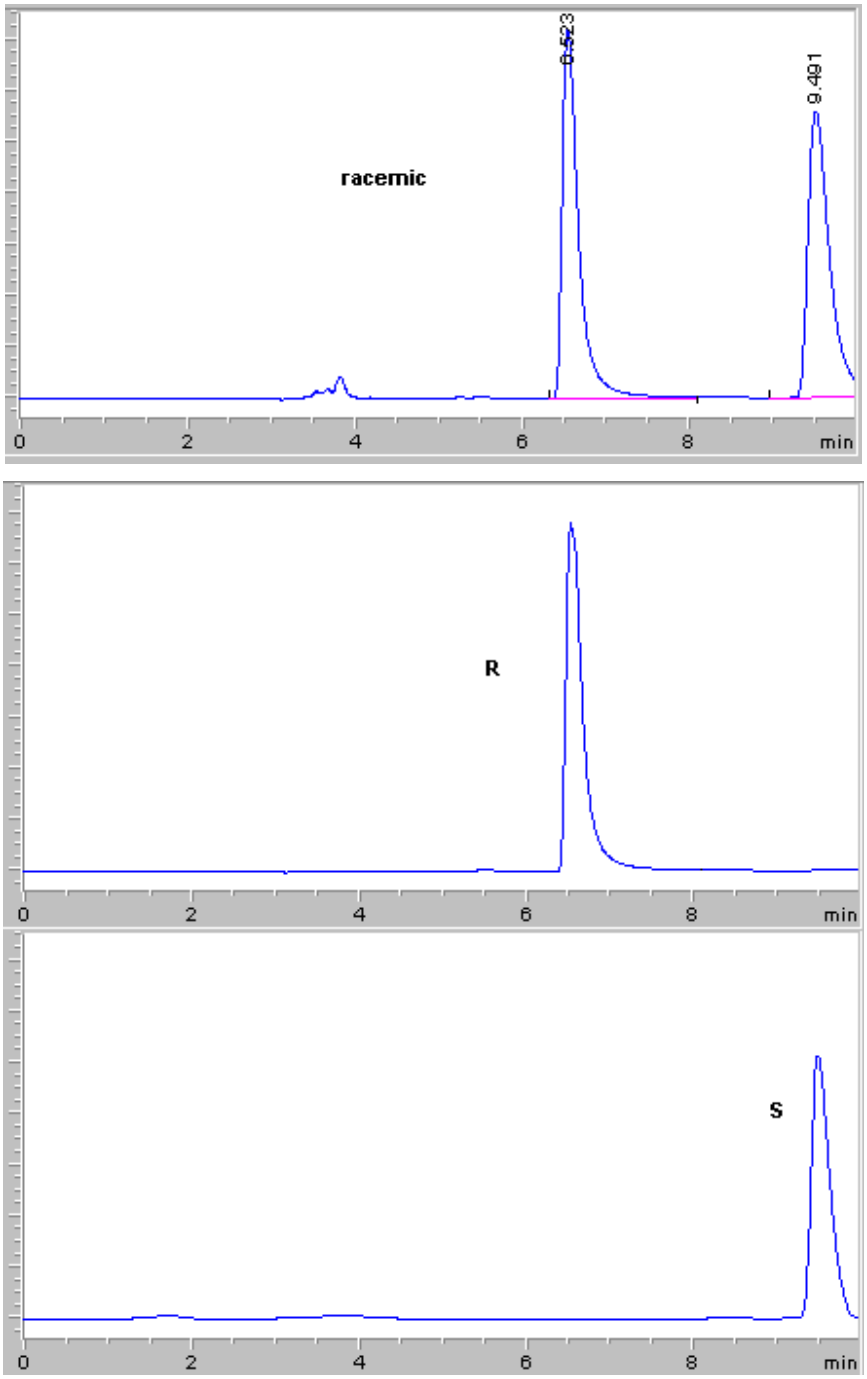

NH2
N

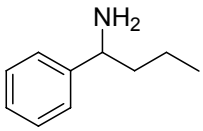

24.046
26.761
5
10
15
20
25
30
min
R
5
10
15
20
25
30
min
S
5
10
15
20
25
30
min

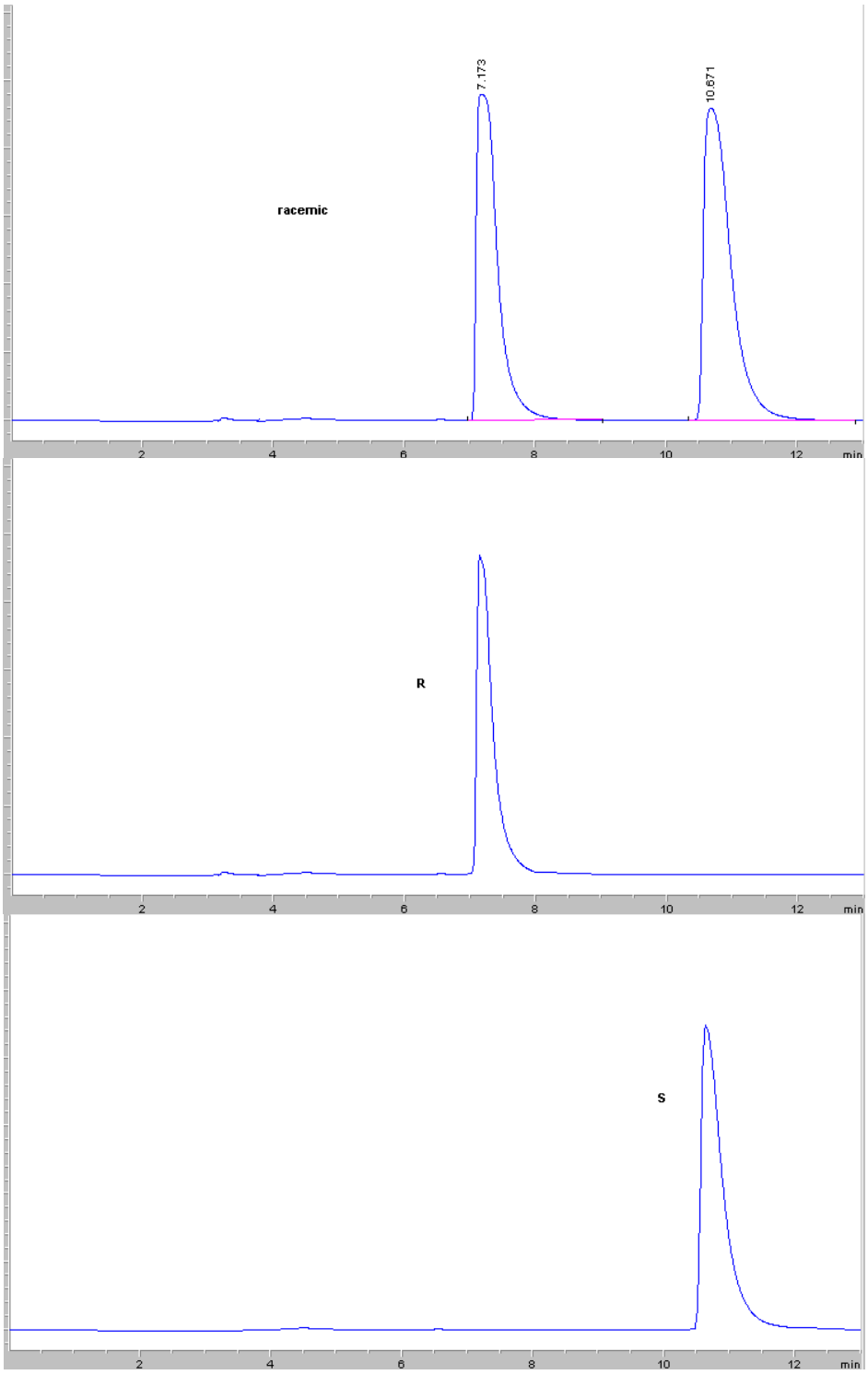

NH2


mixture of R and S
13.057
14.336
0
2
4
6
8
10
12
14
min
R
0
2
4
6
8
10
12
14
min
S
0
2
4
6
8
10
12
14
min


NH2


racemic
9.491
0
2
4
6
8
min
R
0
2
4
6
8
min
S
0
2
4
6
8
min


NH2


racemic
7.173
10.671
2
4
6
8
10
12
min
R
2
4
6
8
10
12
min
S
2
4
6
8
10
12
min