Supporting information

New fluoroethyl phenylalanine analogues as potential LAT-1 targeting PET tracers for glioblastoma

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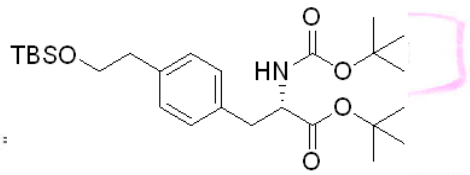
Chiral purity analysis of compound **3** & **4**

Chromatographic conditions for **3** & **4**:

Diacel Chiralpak IA, 250 x 4.6 mm, particle size 5 µm. Column temperature was set at 35 °C. The chromatographic run was performed isocratically with a mixture of *n*-Hexane/EtOH(abs.) (97/3) and a run length of 30 at a flow rate of 1 mL/min.

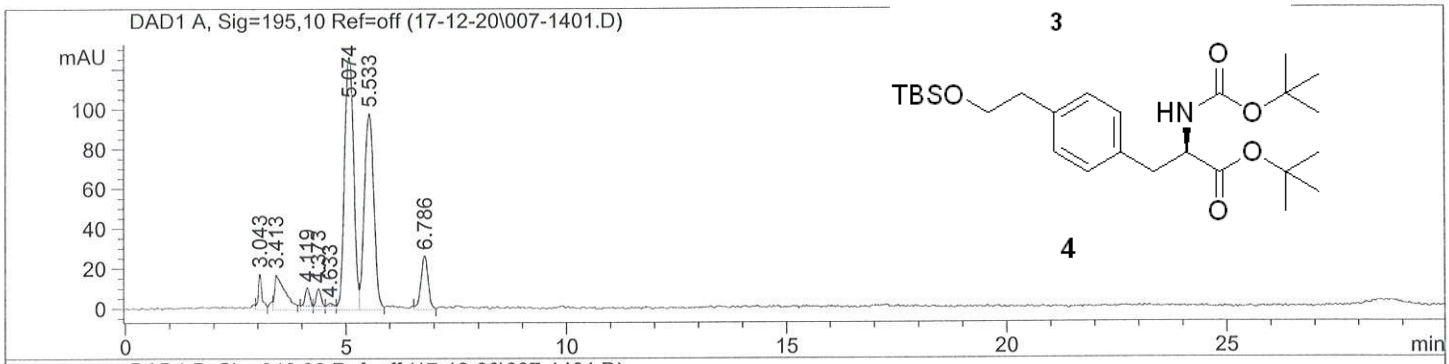
```
==================================================================
Injection Date  : 12/20/2017 5:45:35 PM        Seq. Line :  14
Sample Name     : G                             Location : Vial 7
Acq. Operator   : Jan Goeman                        Inj :   1
                                               Inj Volume : Inj prog

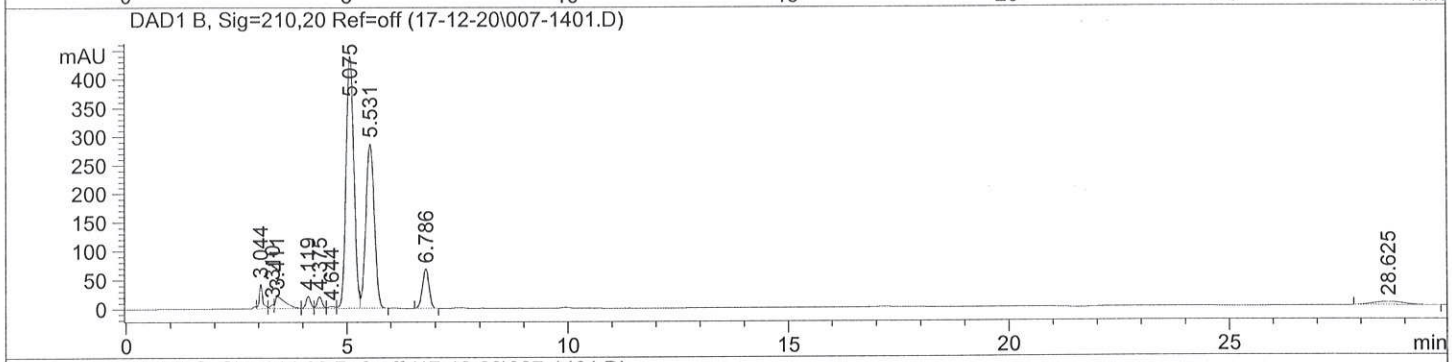
Sequence File   : C:\HPCHEM\2\SEQUENCE\TEMP2.S
Method          : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed    : 4/11/2016 11:58:26 AM by Jan Goeman
Chirale kolom .....
30' isocratisch n-Hexaan-EtOH 97:3
==================================================================
```

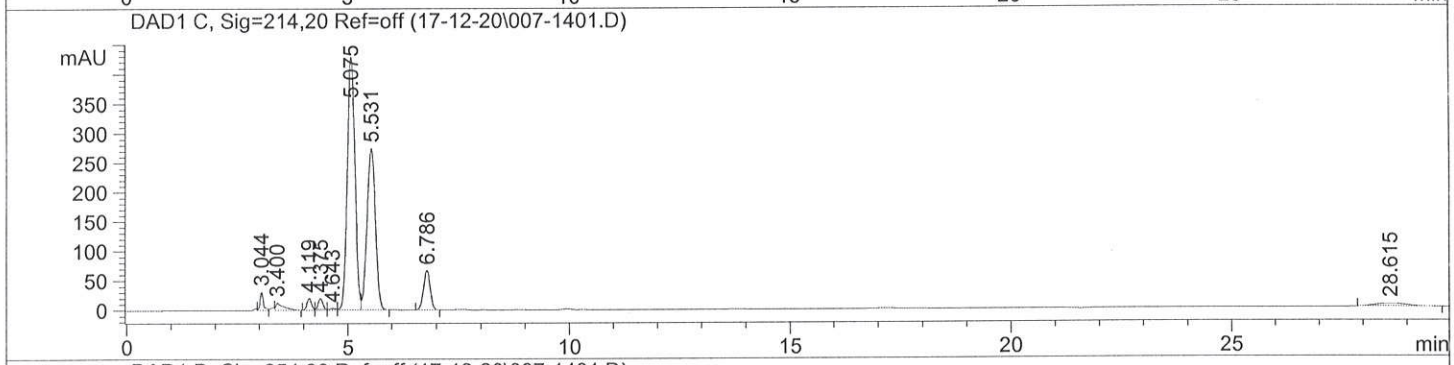


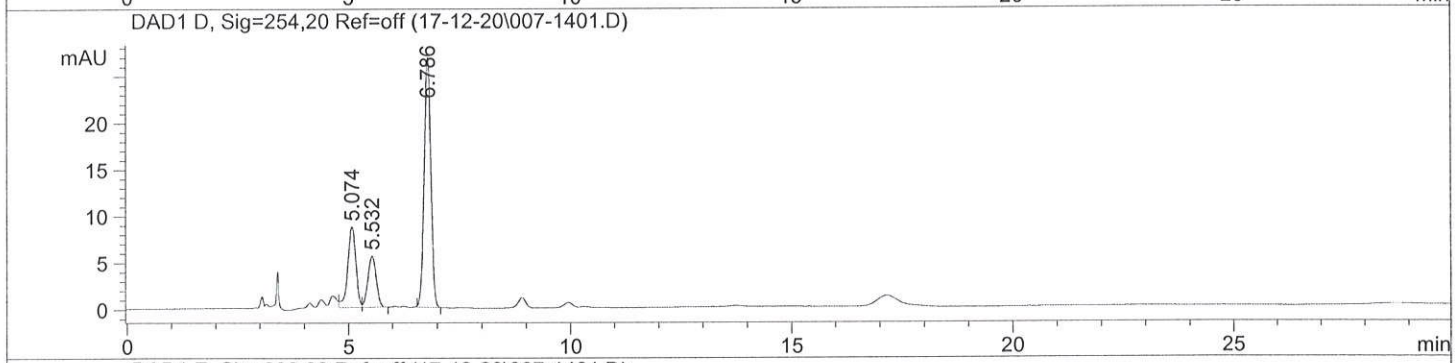
3

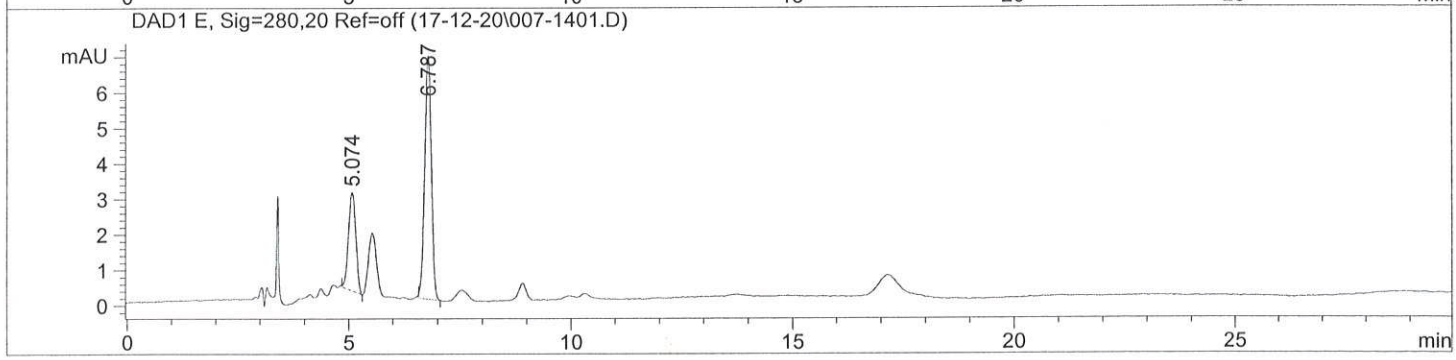
4











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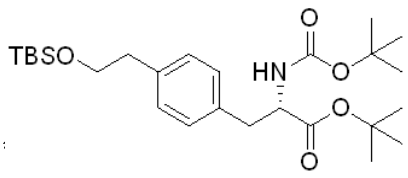
Sample Name     : 13                           Location : Vial 75
Acq. Operator   : Jan Goeman                       Inj :   1
                                               Inj Volume : Inj prog

Sequence File   : C:\HPCHEM\2\SEQUENCE\TEMP2.S
Method          : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed    : 4/11/2016 11:58:26 AM by Jan Goeman
Chirale kolom .....................
30' isocratisch n-Hexaan-EtOH 97:3
```

Chiral HPLC analysis
Sample: cpd. 3



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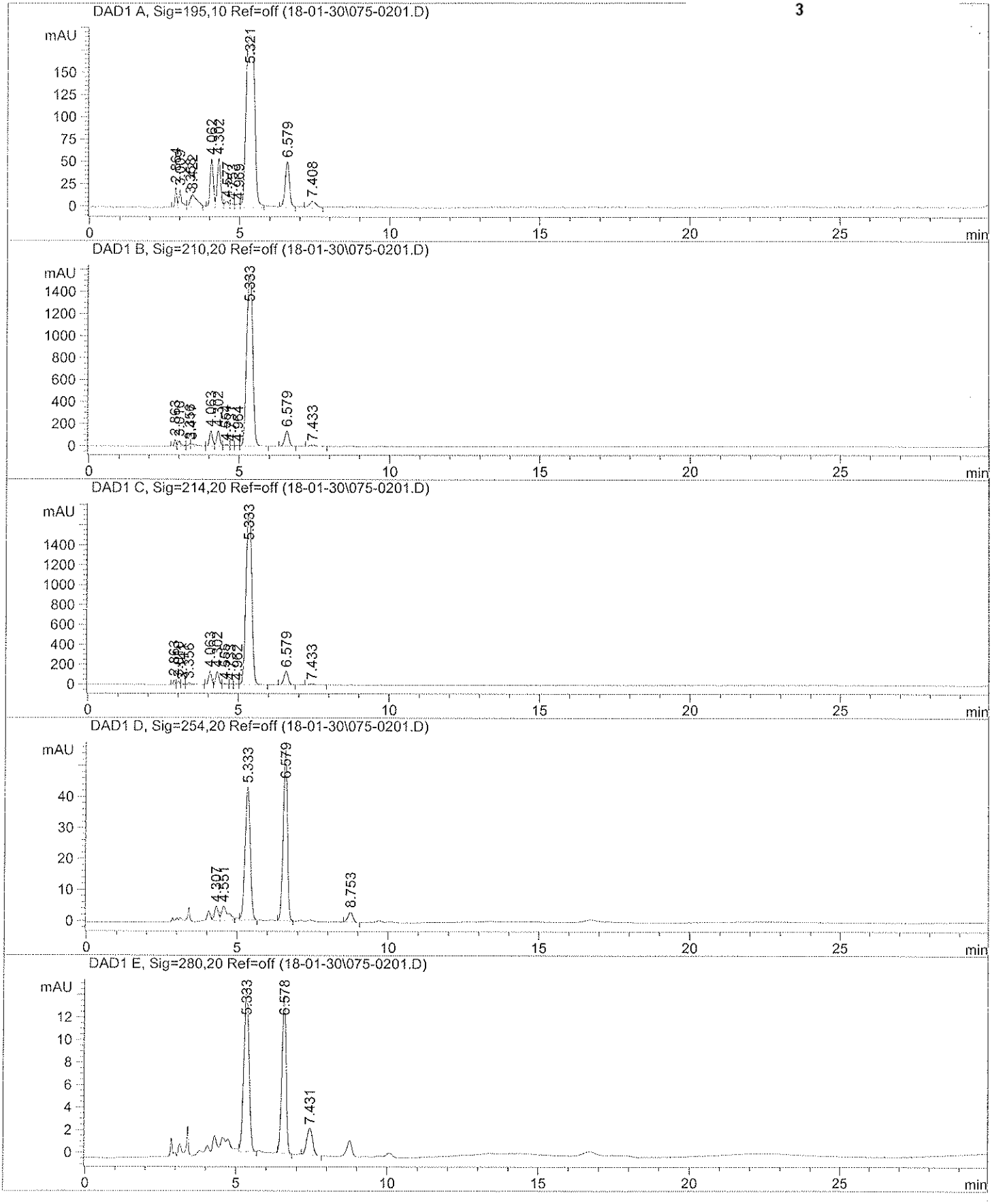
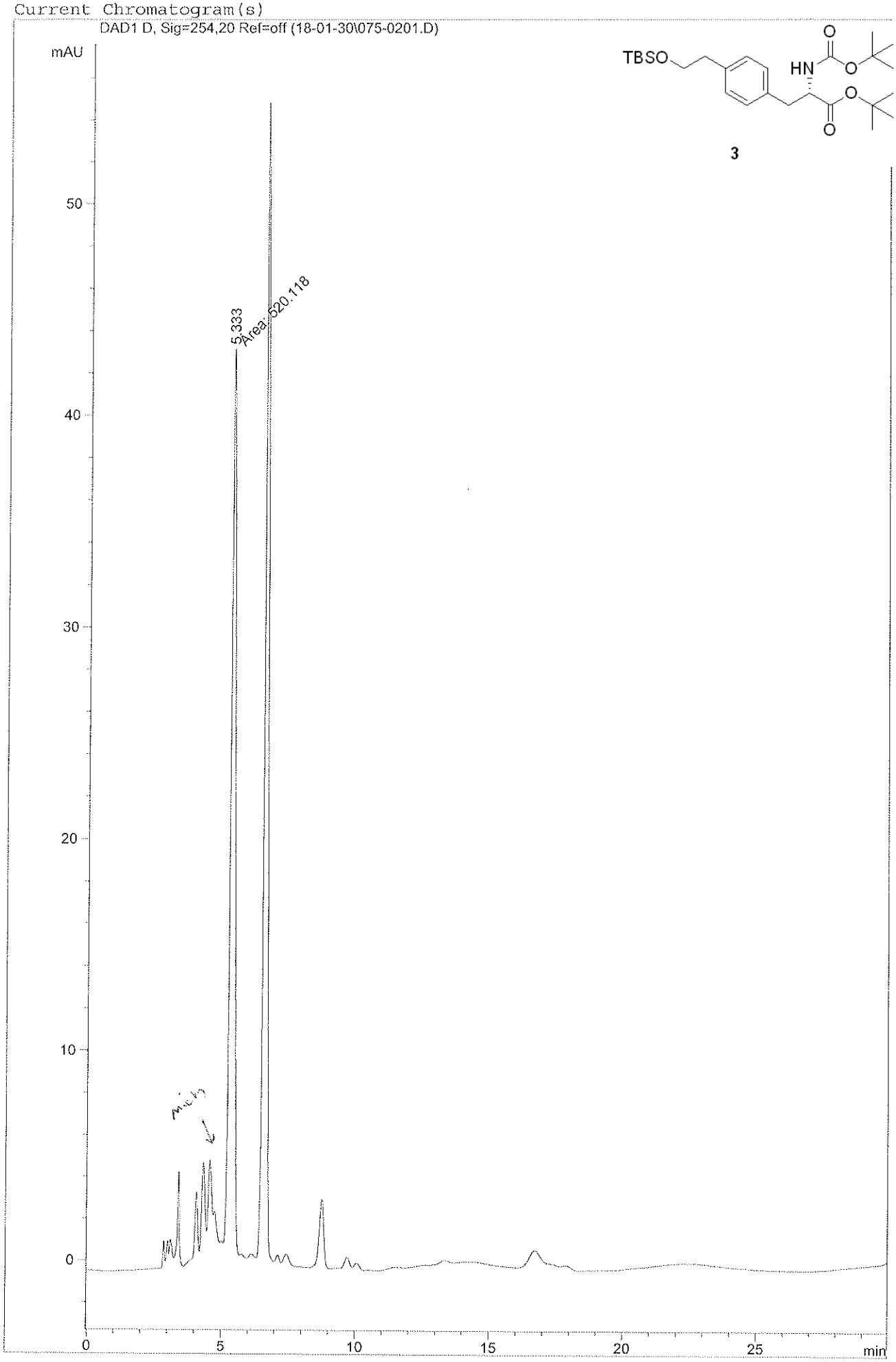


Current Chromatogram(s)

DAD1 D, Sig=254,20 Ref=off (18-01-30\075-0201.D)

3



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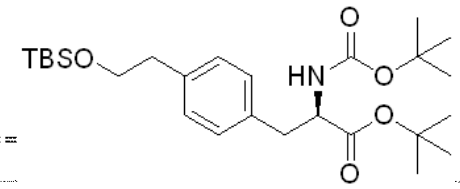
Sample Name      : 14                           Location : Vial 76
Acq. Operator    : Jan Goeman                       Inj :   1
                                                Inj Volume : Inj prog

Sequence File    : C:\HPCHEM\2\SEQUENCE\TEMP2.S
Method           : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed     : 4/11/2016 11:58:26 AM by Jan Goeman
Chirale kolom .....................
30' isocratisch n-Hexaan-EtOH 97:3
```

Chiral HPLC analysis:
Sample: cpd. 4



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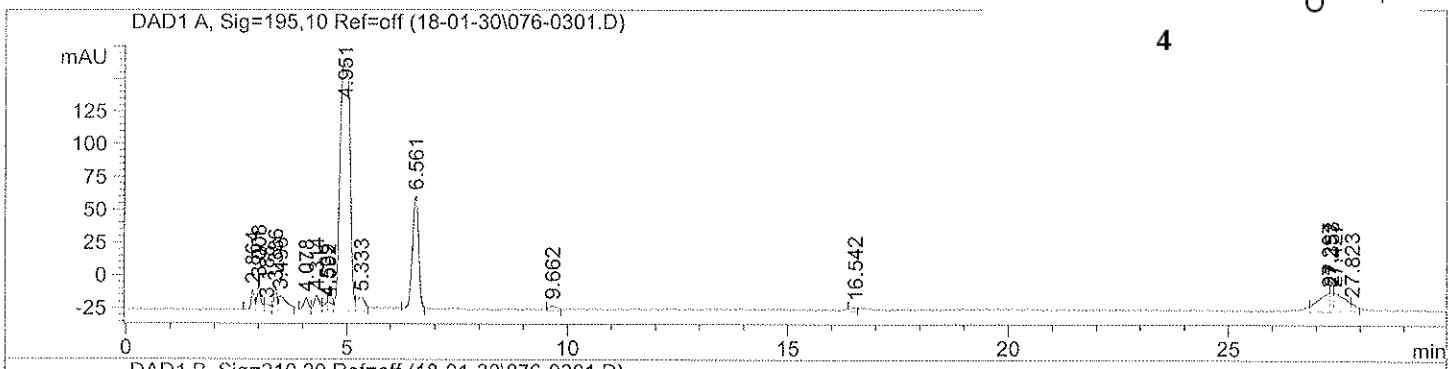
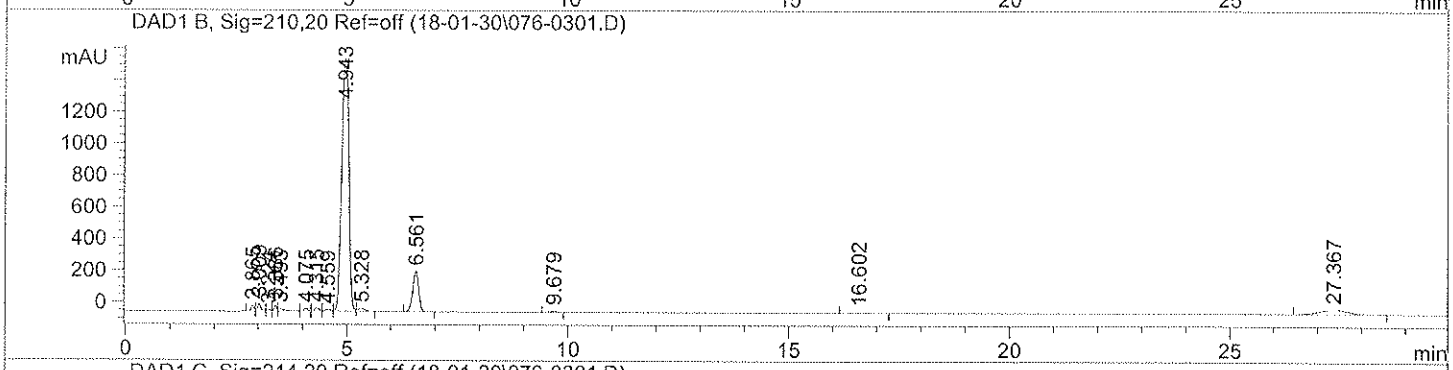
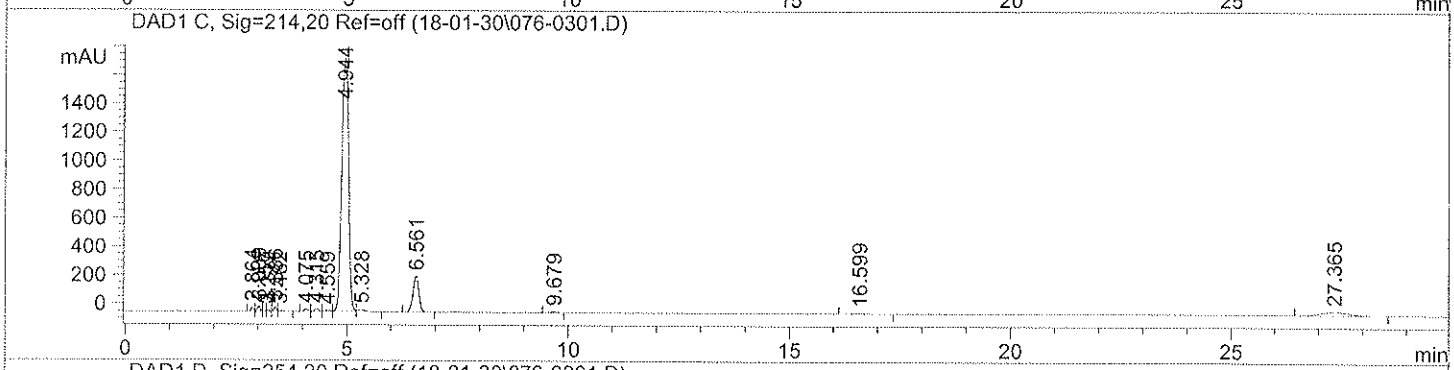
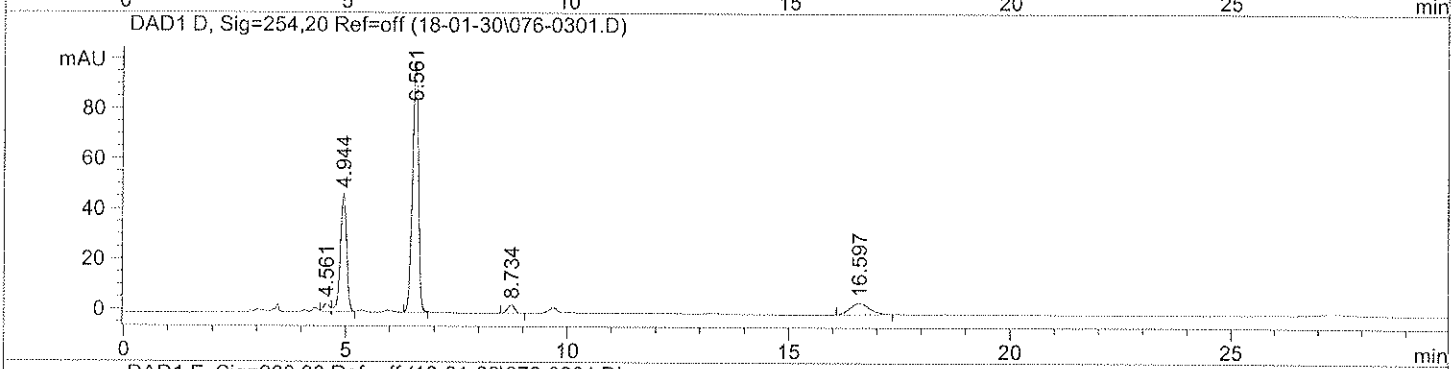
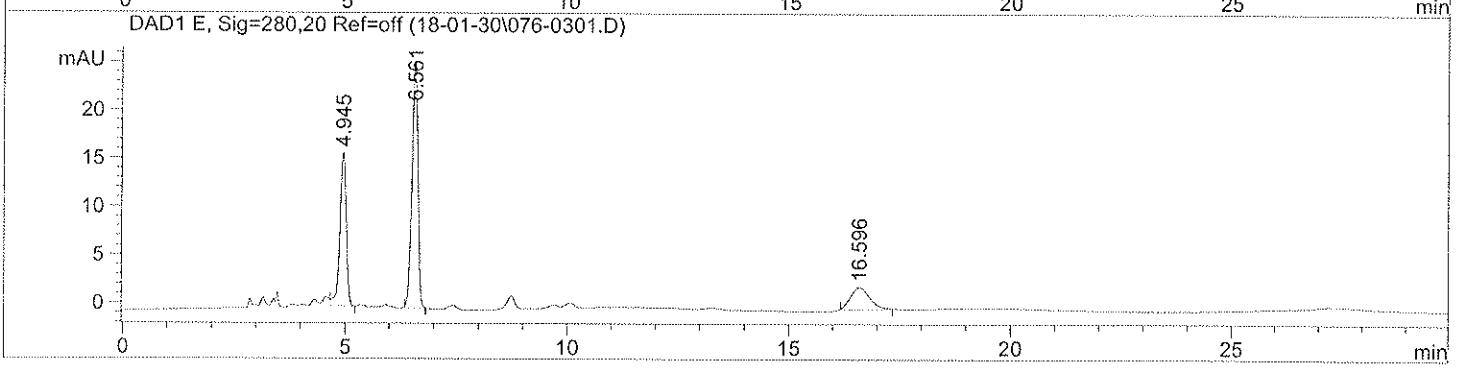




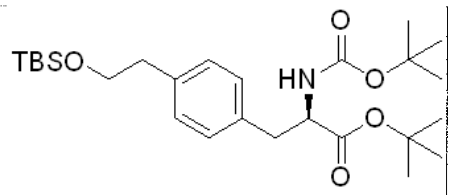






Current Chromatogram(s)

DAD1 D, Sig=254,20 Ref=off (18-01-30\076-0301.D)



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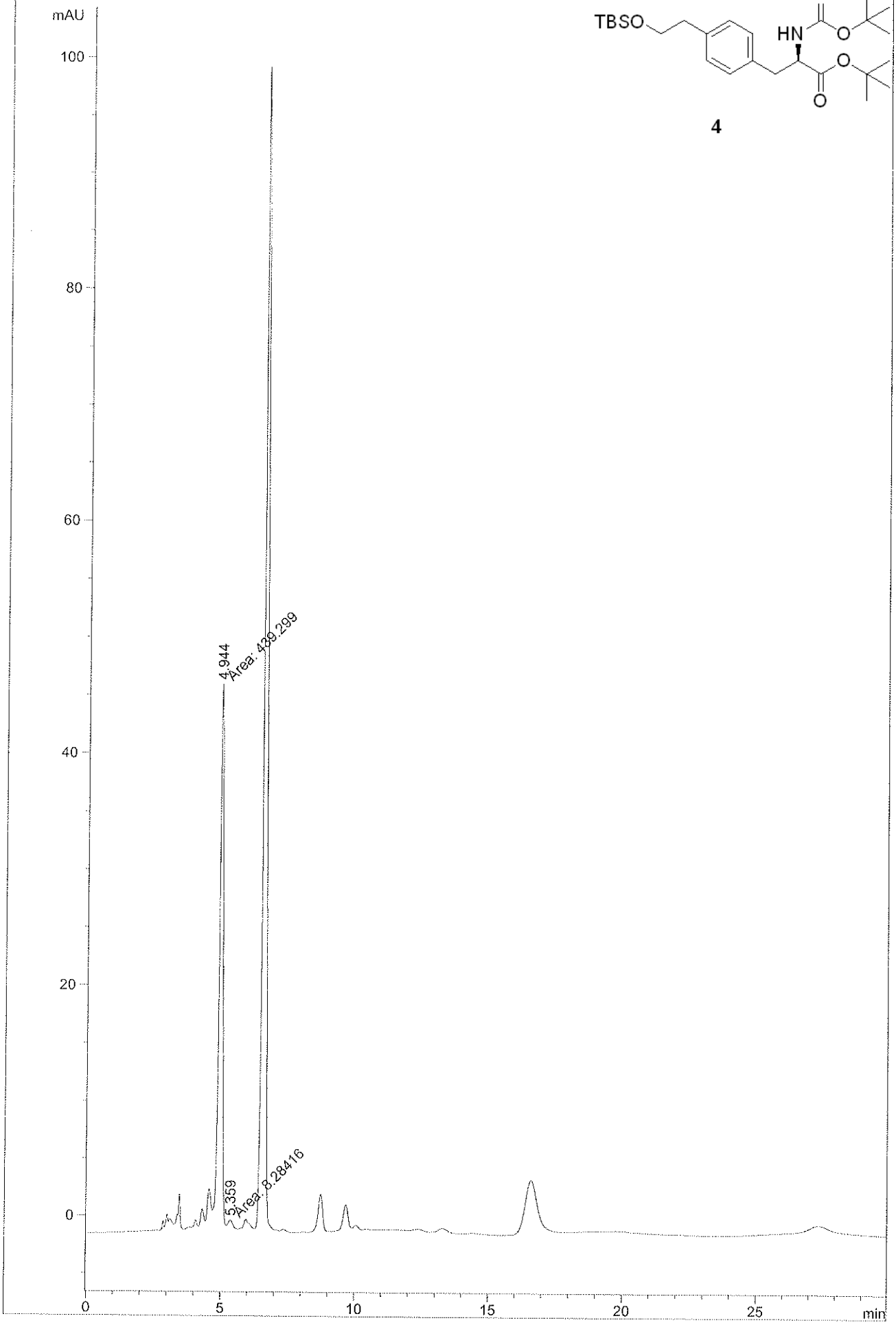

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Sample Name     : 14                             Location : Vial 76
Acq. Operator   : Jan Goeman                        Inj :   1
Acq. Instrument : Instrument 2                  Inj Volume : Inj prog
Acq. Method     : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed    : 4/11/2016 11:58:26 AM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\2CD30-70.M
Last changed    : 1/26/2018 2:43:53 PM by Jan Goeman
                  (modified after loading)
kolom 2
10' Spoelen met 30:70
pomp 1ml/min, geen MS
==============================================================================
                          Area Percent Report
==============================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000


Signal 1: DAD1 A, Sig=195,10 Ref=off


Signal 2: DAD1 B, Sig=210,20 Ref=off


Signal 3: DAD1 C, Sig=214,20 Ref=off


Signal 4: DAD1 D, Sig=254,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.944 MM    0.1584  439.29868   46.22877  98.1491
   2   5.359 MM    0.2010    8.28416 6.86988e-1   1.8509

Totals :                   447.58283   46.91576


Signal 5: DAD1 E, Sig=280,20 Ref=off

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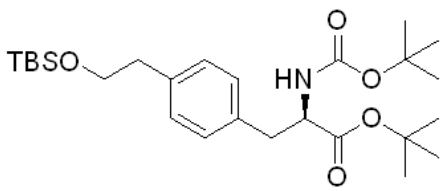


4

Sample: cpd. 4

Chiral purity analysis of compound **9** & **10**

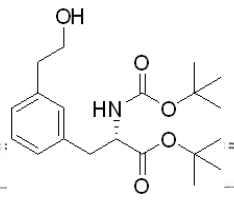
Chromatographic conditions for **9** & **10**:

Diacel Chiralpak IB, 250 x 4.6 mm, particle size 5 µm. Column temperature was set at 35 °C. The chromatographic run was performed isocratically with a mixture of *n*-Hexane/EtOH(abs.) (97/3) and a run length of 30 at a flow rate of 1 mL/min.

Injection Date : 12/22/2017 2:06:37 PM Seq. Line : 2
Sample Name : L Location : Vial 12
Acq. Operator : Jan Goeman Inj : 1
Inj Volume : Inj prog

Sequence File : C:\HPCHEM\2\SEQUENCE\TEMP2.S
Method : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed : 4/11/2016 11:58:26 AM by Jan Goeman
Chirale kolom .....
30' isocratisch n-Hexaan-EtOH 97:3

Optimization of chiral HPLC
Sample: cpd. 9 & 10



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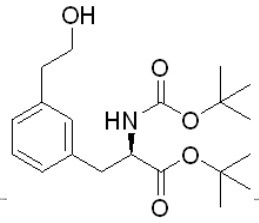

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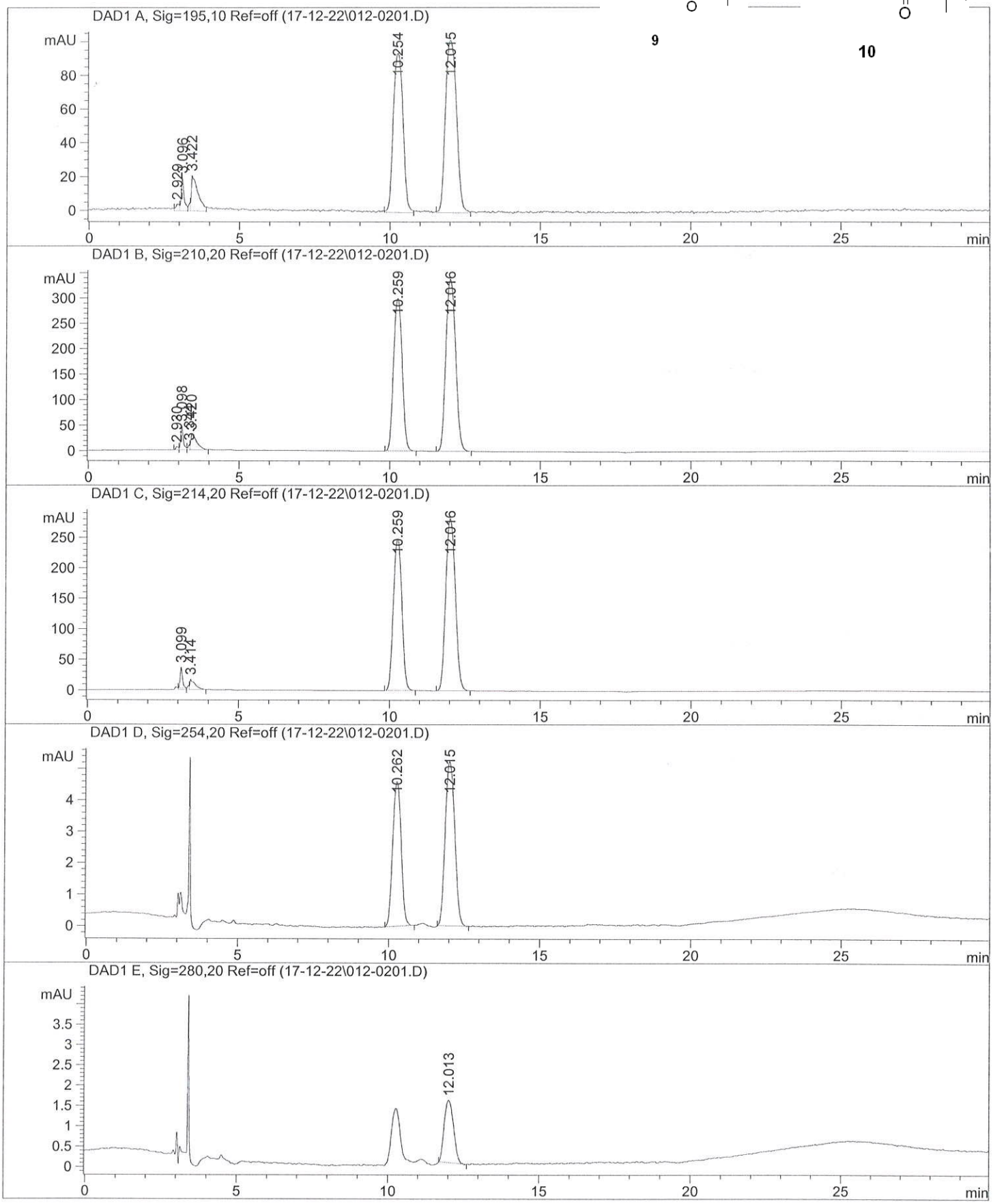

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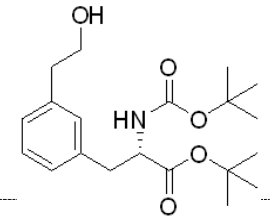
Injection Date  : 12/22/2017 7:39:08 PM          Seq. Line :  12
Sample Name     : 23                             Location : Vial 56
Acq. Operator   : Jan Goeman                        Inj :  1
                                                 Inj Volume : Inj prog

Sequence File   : C:\HPCHEM\2\SEQUENCE\TEMP2.S
Method          : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed    : 4/11/2016 11:58:26 AM by Jan Goeman
Chirale kolom .....................
30' isocratisch n-Hexaan-EtOH 97:3
==========================================================================
```

Chiral HPLC analysis
Compound 9



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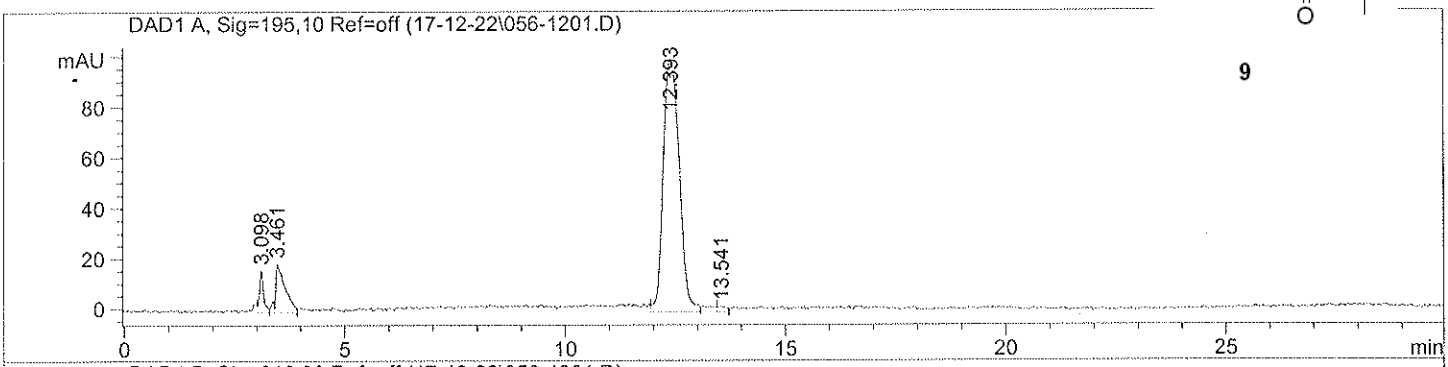
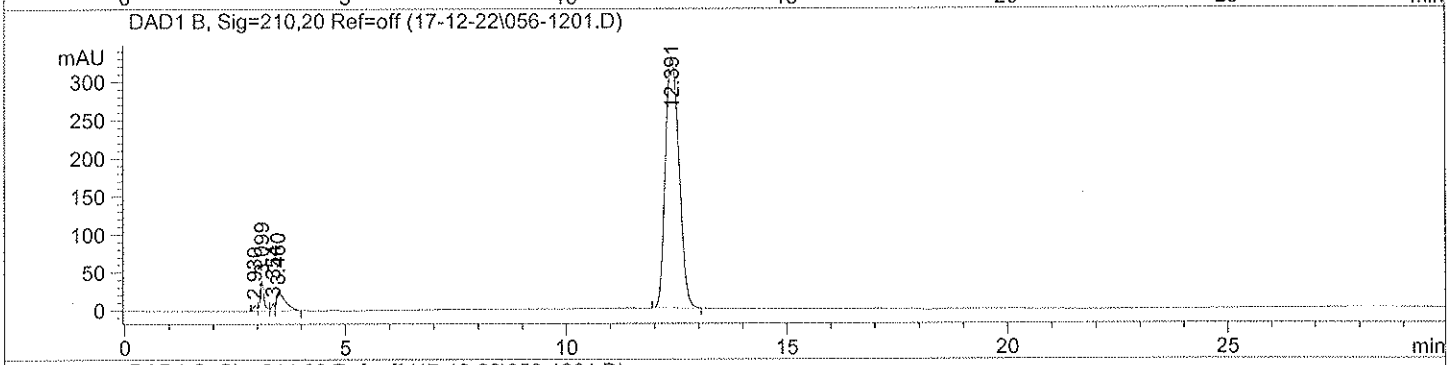
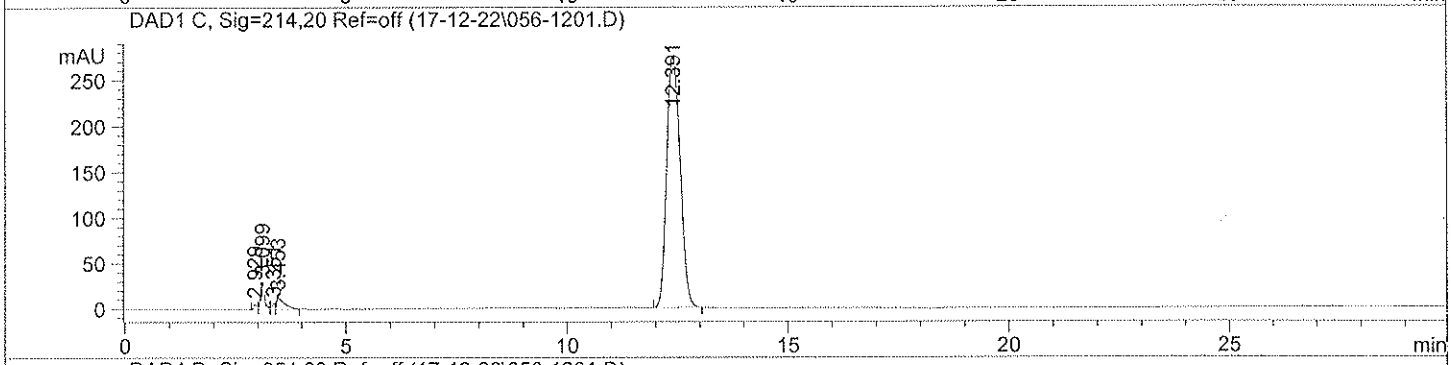
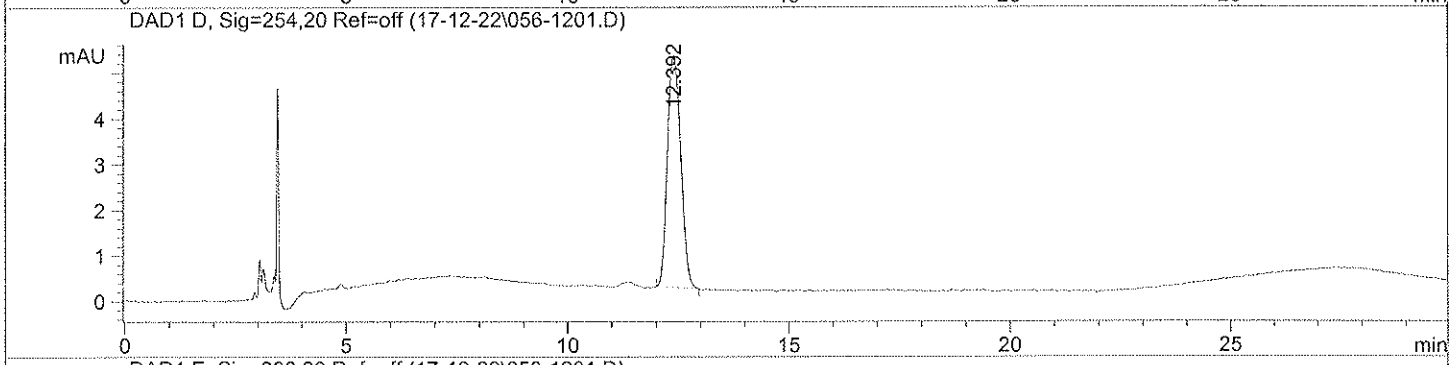
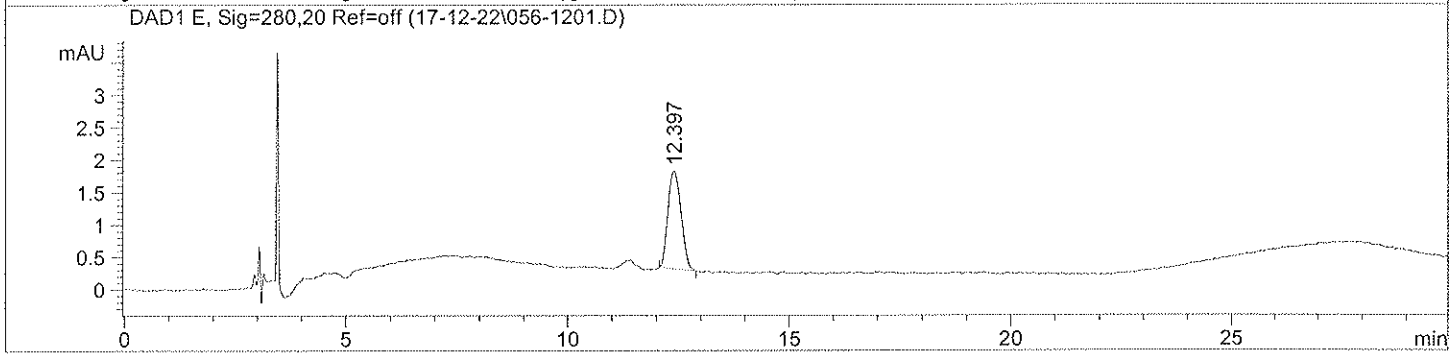


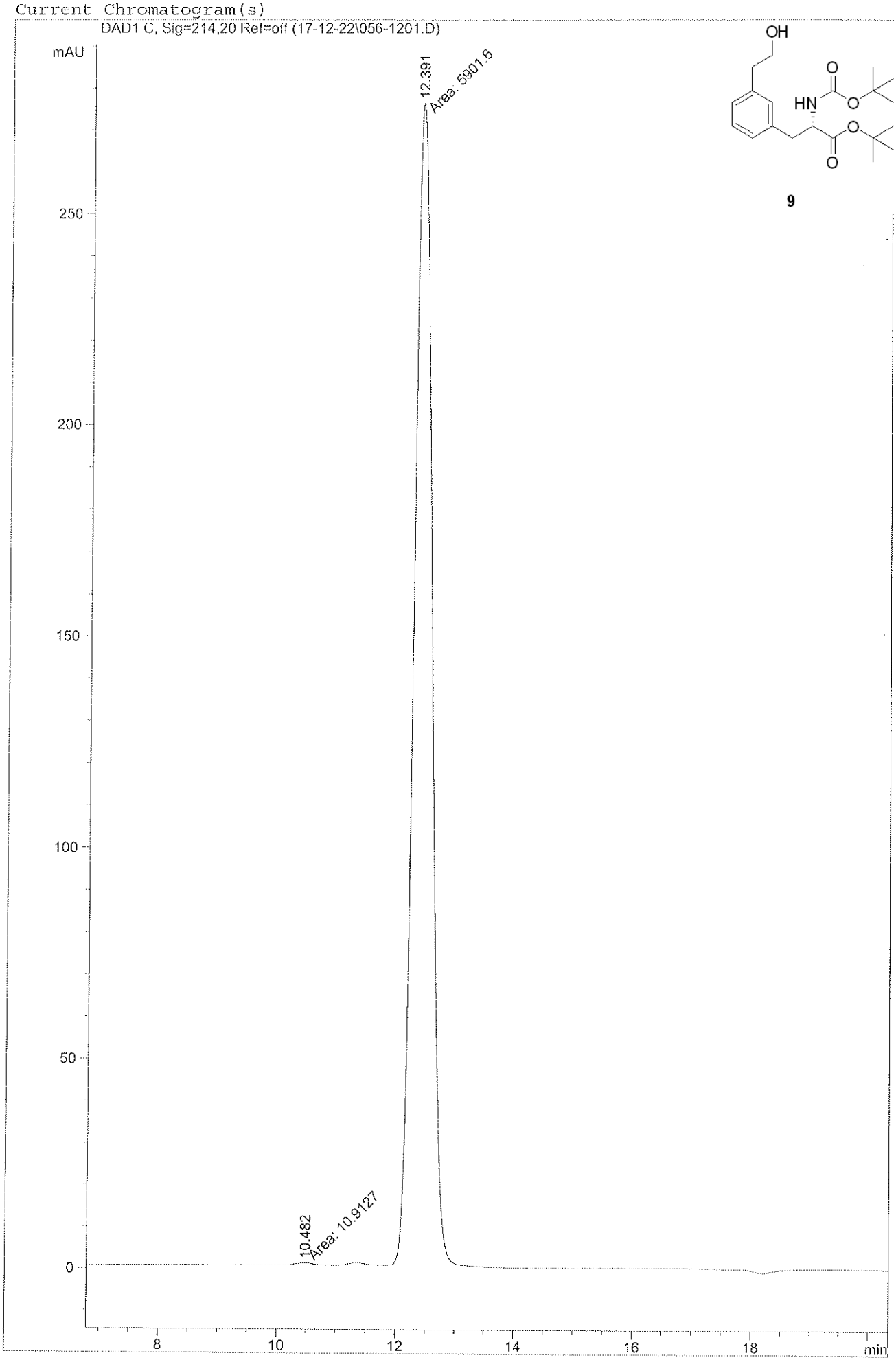








Current Chromatogram(s)



9

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Acq. Operator   : Jan Goeman                          Inj :  1
Acq. Instrument : Instrument 2                 Inj Volume : Inj prog
Acq. Method     : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed    : 4/11/2016 11:58:26 AM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\2CD30-70.M
Last changed    : 1/26/2018 2:43:53 PM by Jan Goeman
                  (modified after loading)
kolom 2
10' Spoelen met 30:70
pomp 1ml/min, geen MS
==============================================================================
                          Area Percent Report
==============================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
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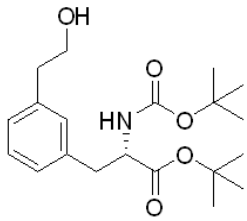

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```
Signal 1: DAD1 C, Sig=214,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
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----|-------|----|-------|----------|----------|--------|
   1   2.929 BV    0.0725   30.39187    6.06415   0.4805
   2   3.099 VV    0.0890  181.64526   30.73150   2.8720
   3   3.351 VV    0.0742   33.01746    6.85152   0.5220
   4   3.453 VB    0.1459  167.21483   14.57481   2.6438
   5  10.482 MM    0.3339   10.91270 5.44788e-1   0.1725
   6  12.391 MM    0.3567 5901.60303  275.75787  93.3091

Totals :                  6324.78515  334.52464

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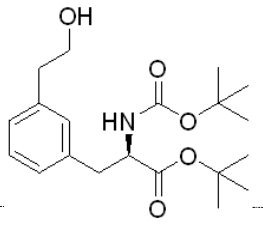
```
Injection Date   : 12/22/2017 8:14:01 PM          Seq. Line :  13
Sample Name      : 24                             Location : Vial 57
Acq. Operator    : Jan Goeman                         Inj :  1
                                                  Inj Volume : Inj prog

Sequence File    : C:\HPCHEM\2\SEQUENCE\TEMP2.S
Method           : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed     : 4/11/2016 11:58:26 AM by Jan Goeman
Chirale kolom .....................
30' isocratisch n-Hexaan-EtOH 97:3
```

Chiral HPLC analysis
Compound 10



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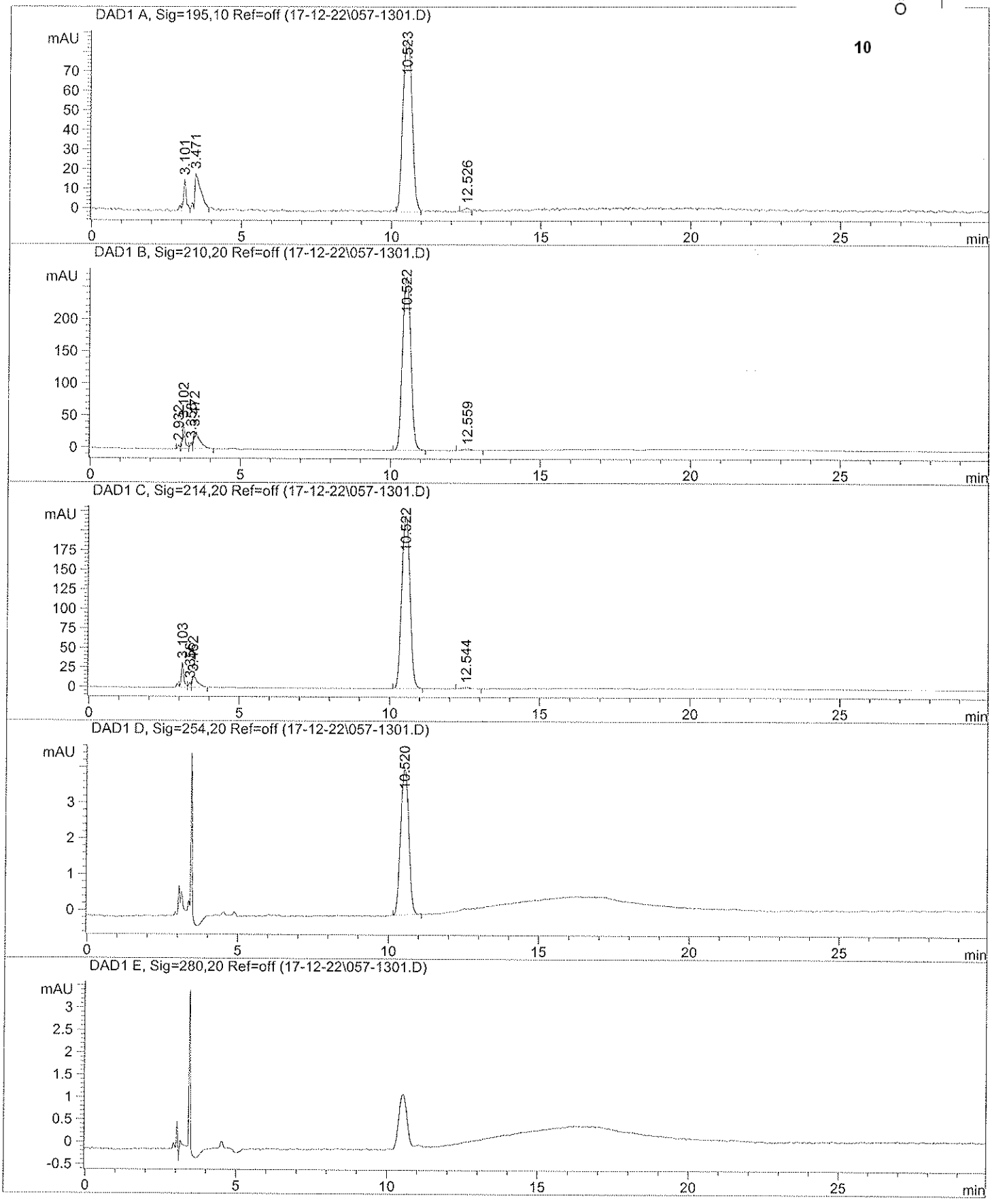
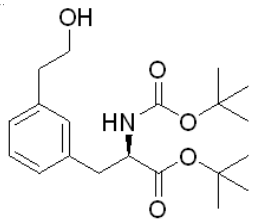


Current Chromatogram(s)

DAD1 C, Sig=214,20 Ref=off (17-12-22\057-1301.D)



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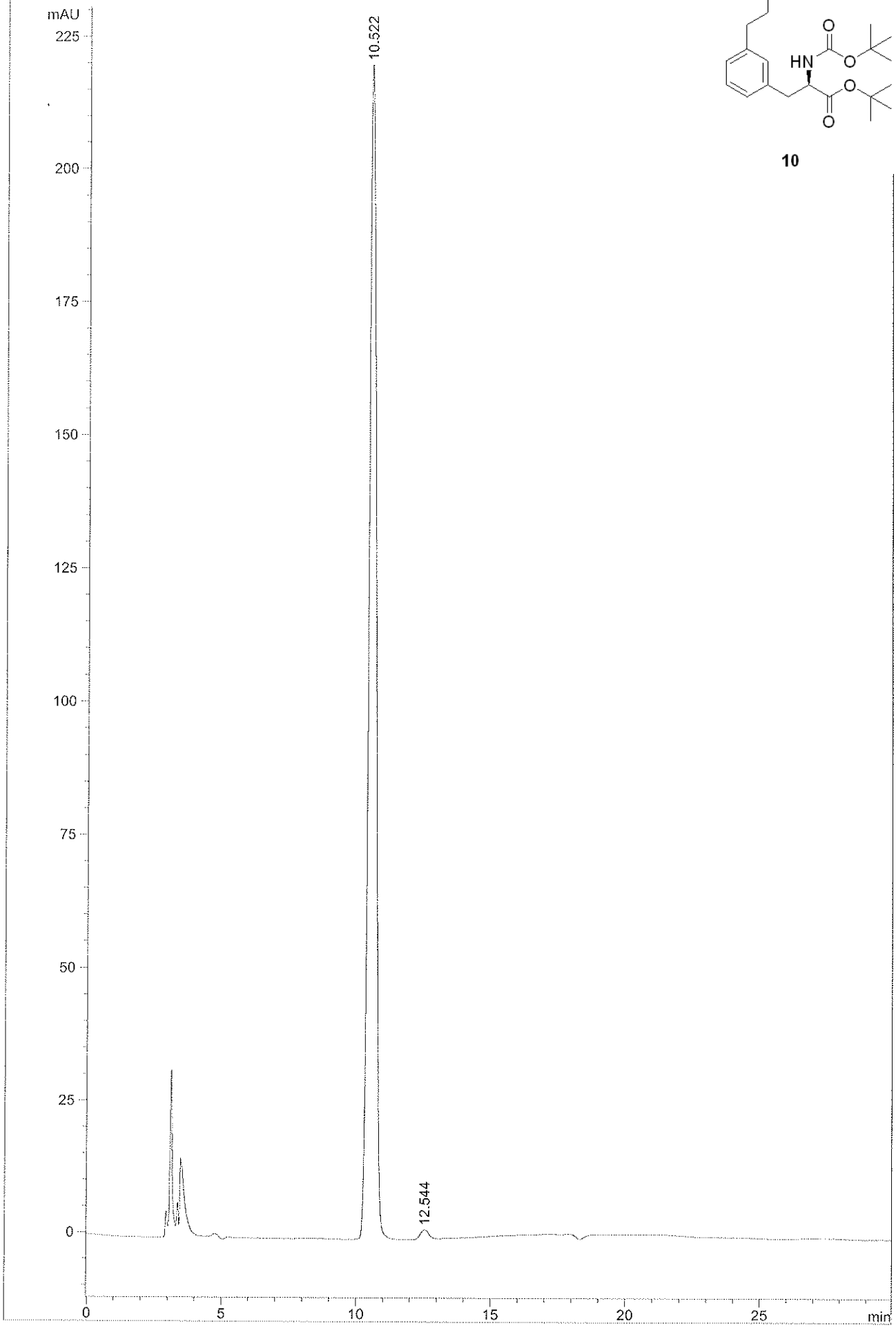

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Sample Name     : 24                              Location : Vial 57
Acq. Operator   : Jan Goeman                          Inj :  1
Acq. Instrument : Instrument 2                  Inj Volume : Inj prog
Acq. Method     : C:\HPCHEM\2\METHODS\AB97-3.M
Last changed    : 4/11/2016 11:58:26 AM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\2CD30-70.M
Last changed    : 1/26/2018 2:43:53 PM by Jan Goeman
                  (modified after loading)
kolom 2
10' Spoelen met 30:70
pomp 1ml/min, geen MS
==============================================================================
                          Area Percent Report
==============================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
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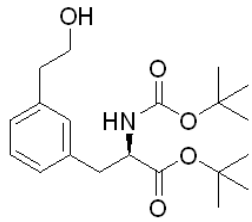

10

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Signal 1: DAD1 C, Sig=214,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
-----|-------|----|-------|----------|----------|--------|
   1  10.522 BB    0.3103 4241.71045  220.65605  99.1330
   2  12.544 PB    0.3225   37.09894    1.75574   0.8670

Totals :                  4278.80939  222.41179

==============================================================================
                          *** End of Report ***
```

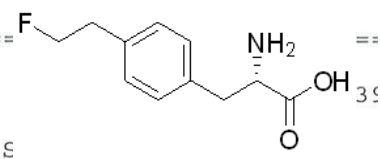
Chiral purity analysis of compound **15** & **16**

Chromatographic conditions for **15** & **16**:

Chiral capillary electrophoresis (CE) employed a 64.5 cm capillary (50 µm diameter). The electrolyte used consisted of 25 mM phosphate buffer pH 2.5 + 5% HS-gammaCD chiral modifier. Analysis time was 40 min at -20 kV.

Data File D:\DATA\18-04-18\039-0401.D

Injection Date : 4/18/18 9:35:04 PM
Sample Name : 19+21
Acq. Operator : Jan Goeman
Sequence File : C:\HPCHEM\1\SEQUENCE\TEMP.S
Method : C:\HPCHEM\1\METHODS\CE\01-4
Last changed : 5/11/11 3:19:59 PM by Jan G
CE met buffer 1
L=.64.5.cm / d=..50.um / pH.2,5. .25...mM / SDS. /...mM / 15.y.CD .5...%



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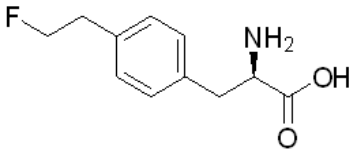
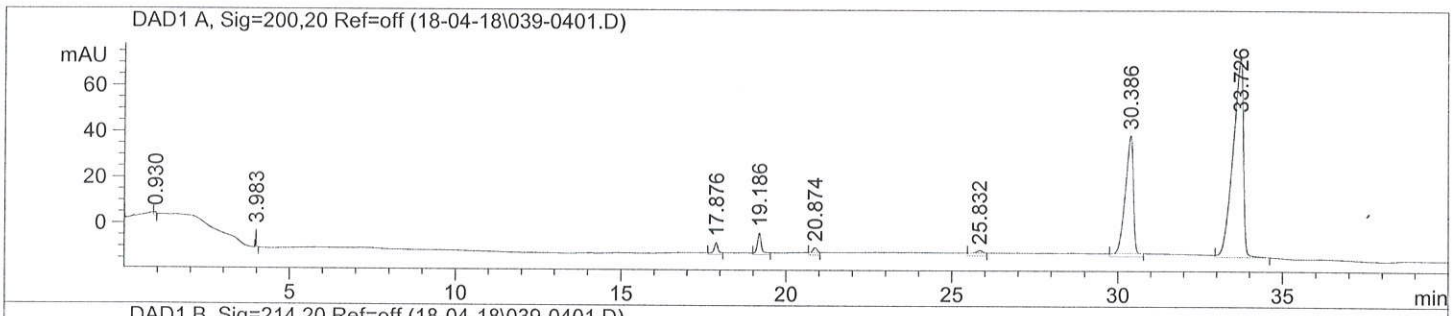
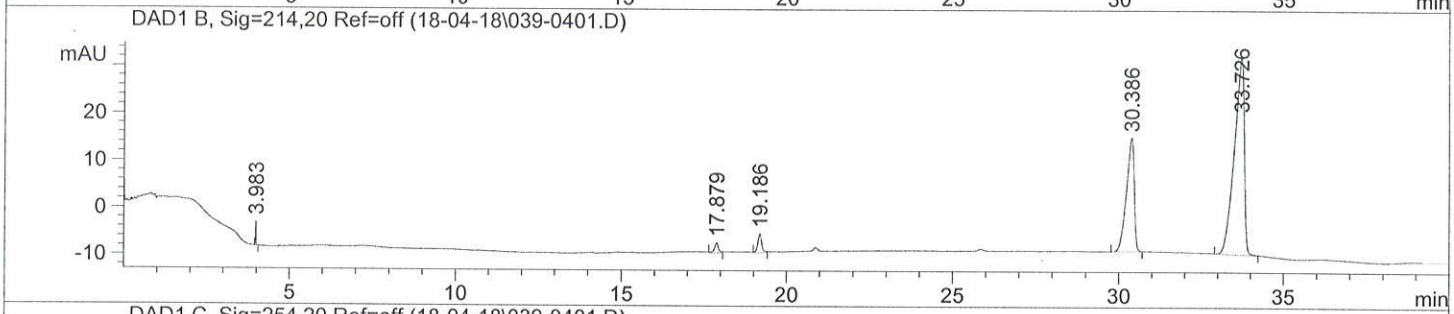
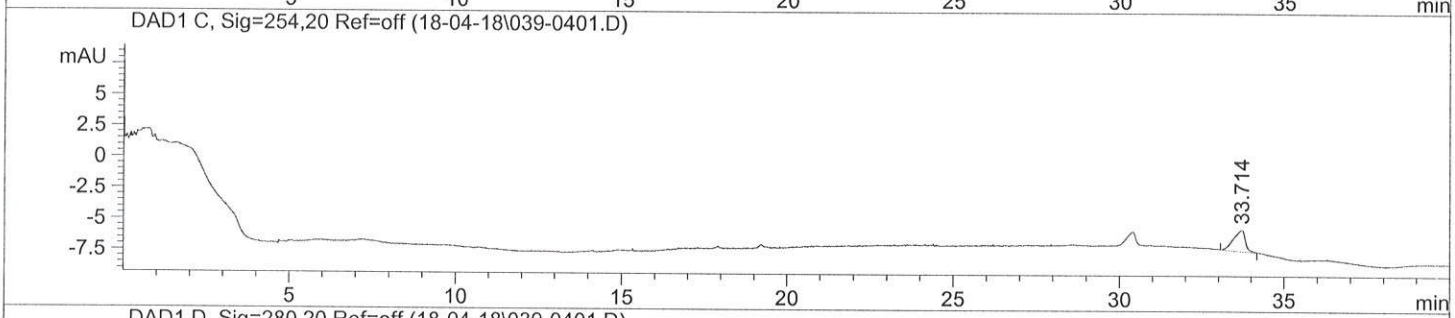


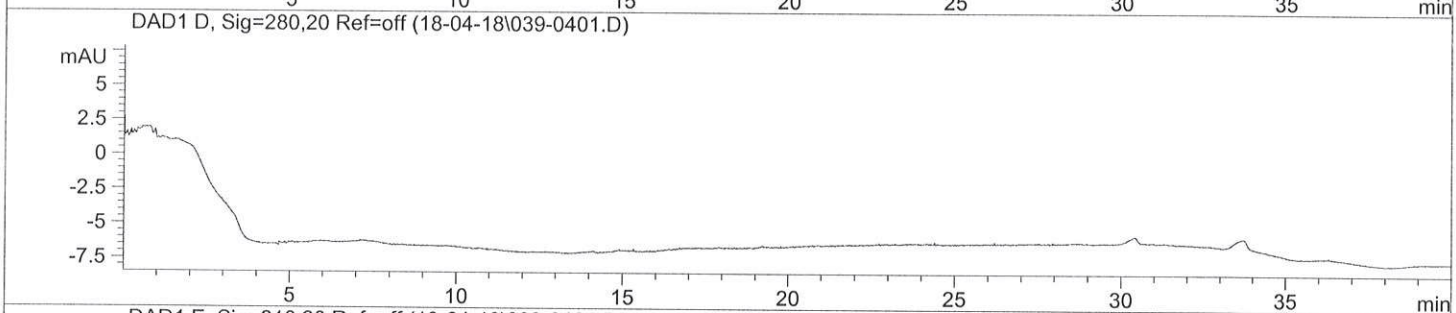
16

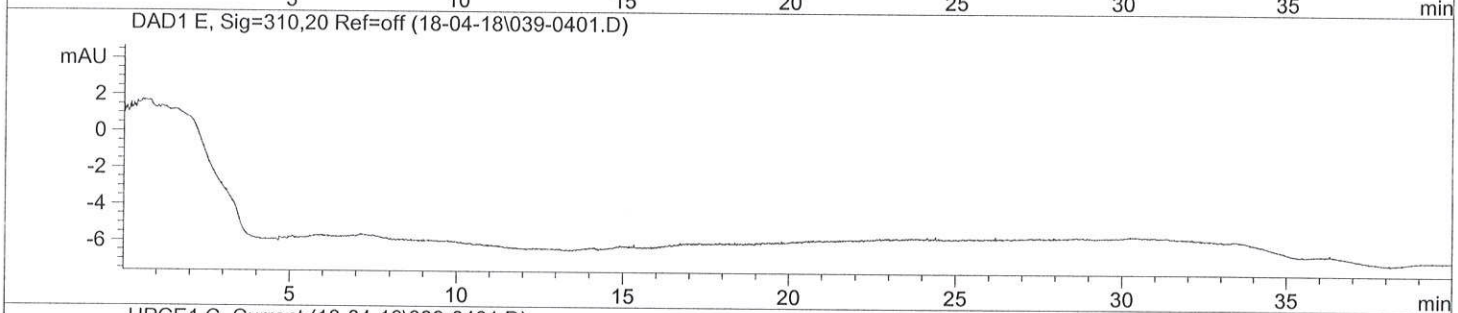
Optimization of CE analysis
Sample: cpd. 15 & 16

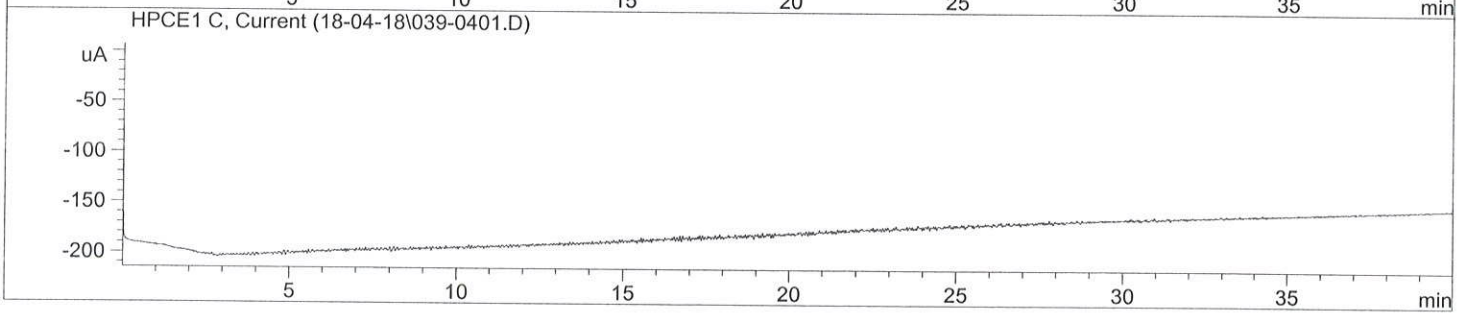




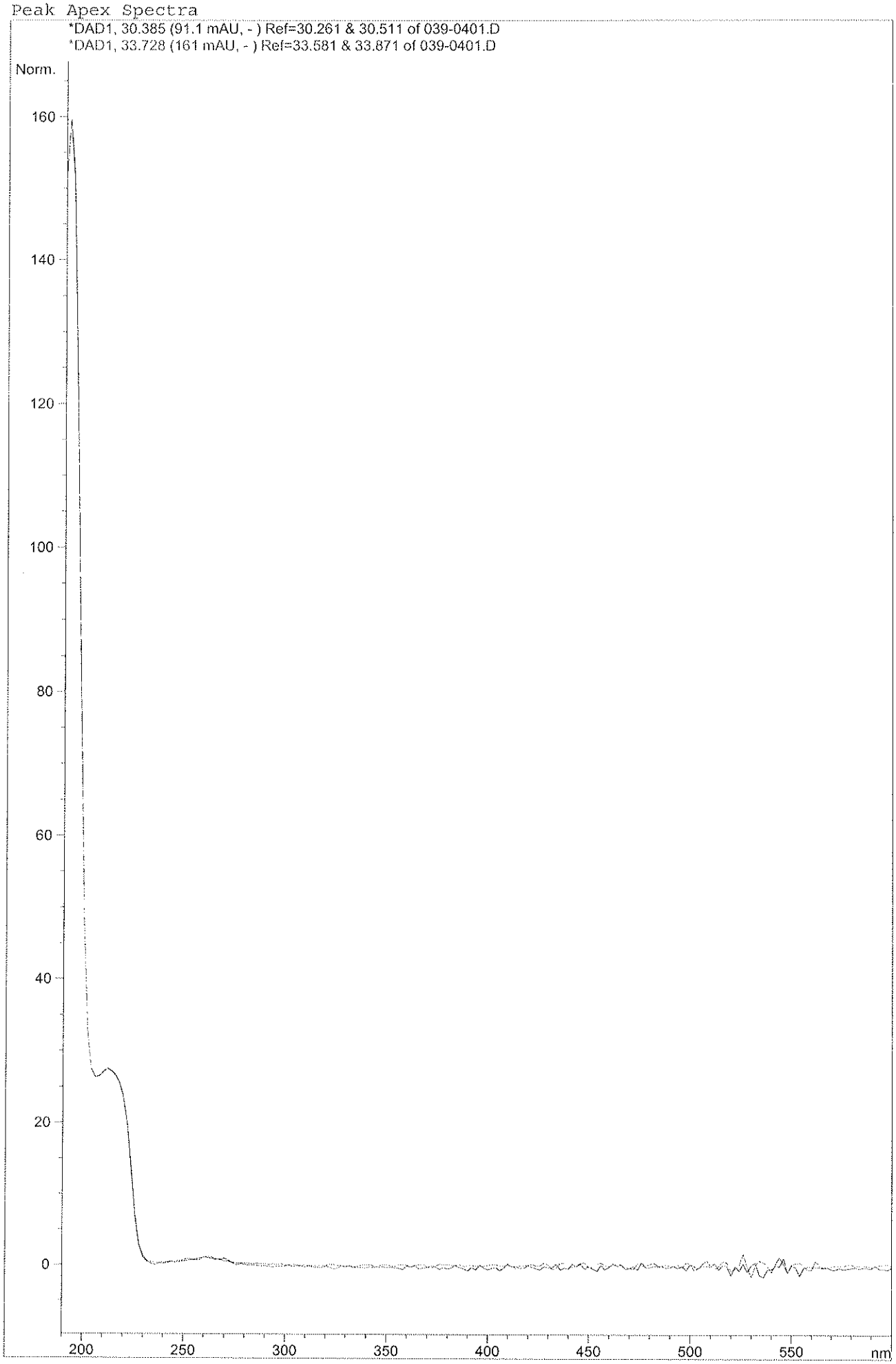






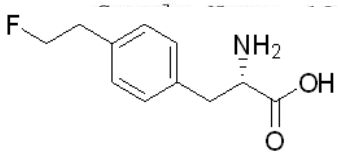


Peak Apex Spectra



Data File D:\DATA\18-04-18\037-0501.D

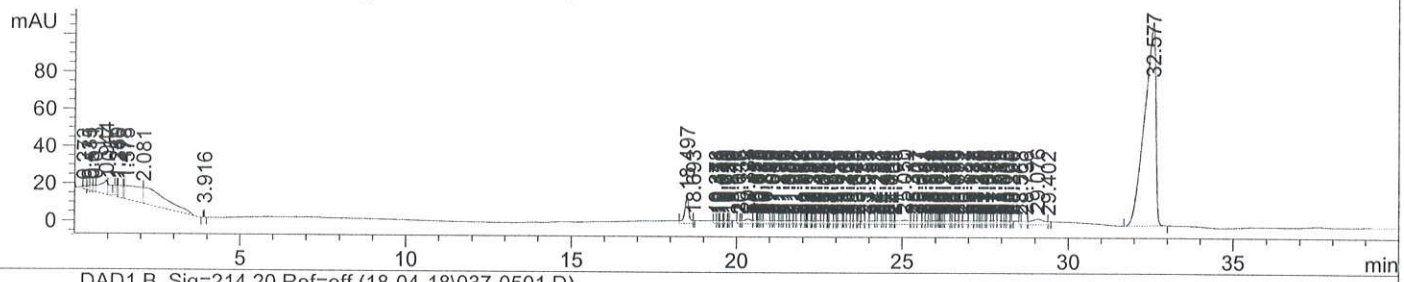
```
==========================================================================
Injection Date  : 4/18/18 10:19:20 PM          Seq. Line :   5
Sample Name     : 19                           Location : Vial 37
Acq. Operator   : Jan Goeman                       Inj :   1
Sequence File   : C:\HPCHEM\1\SEQUENCE\TEMP.S
Method          : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed    : 5/11/11 3:19:59 PM by Jan Goeman
CE met buffer 1
L=64,5.cm / d=..50..um / pH.2,5. .25...mM / SDS.....mM / MS.x.CD ..5...%
==========================================================================
```



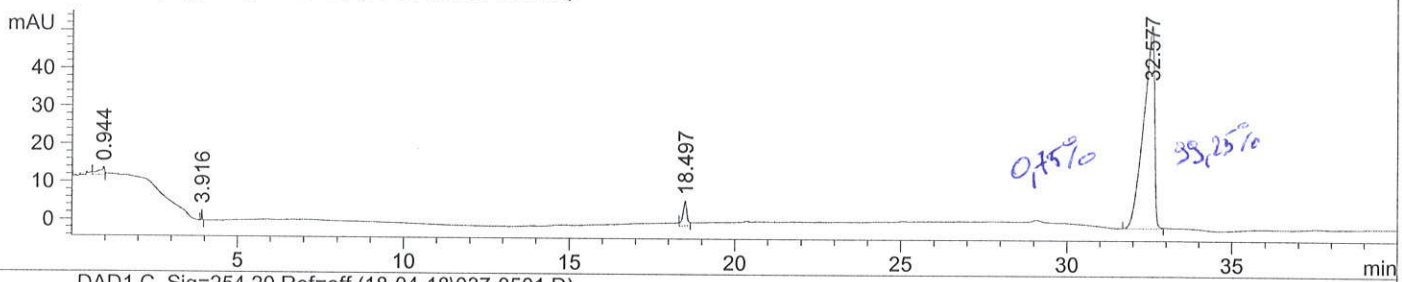
15

Sample: cpd. 15

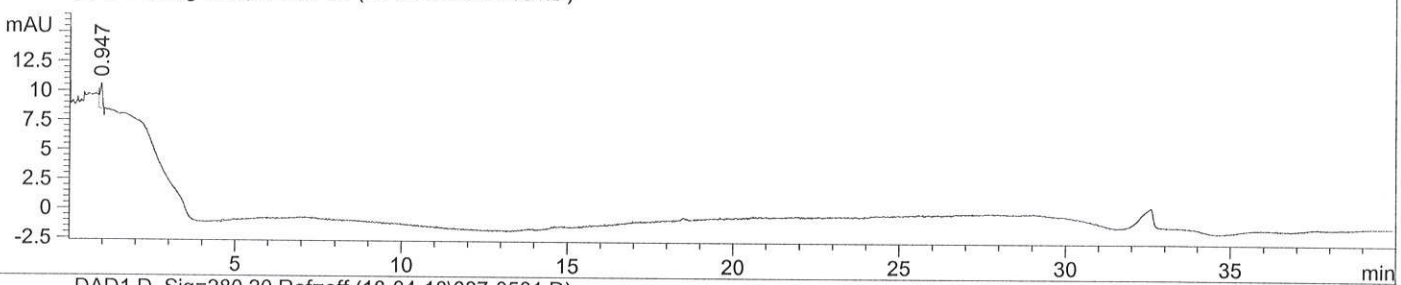
DAD1 A, Sig=200,20 Ref=off (18-04-18\037-0501.D)



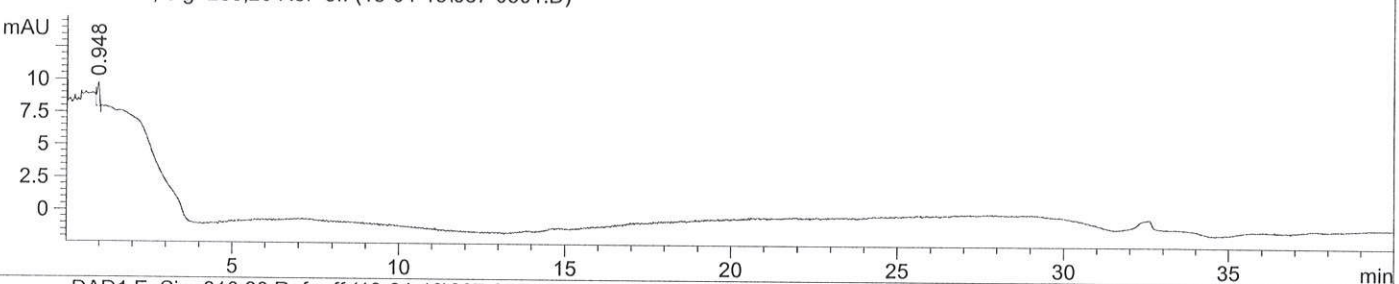
DAD1 B, Sig=214,20 Ref=off (18-04-18\037-0501.D)



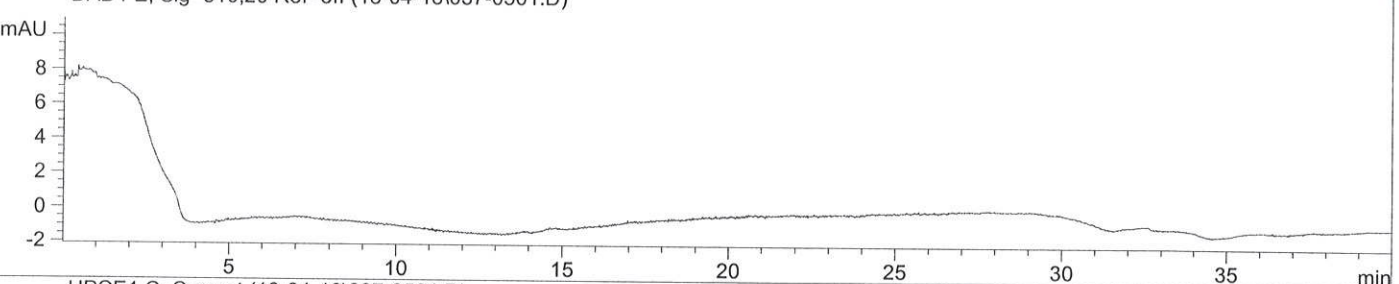
DAD1 C, Sig=254,20 Ref=off (18-04-18\037-0501.D)



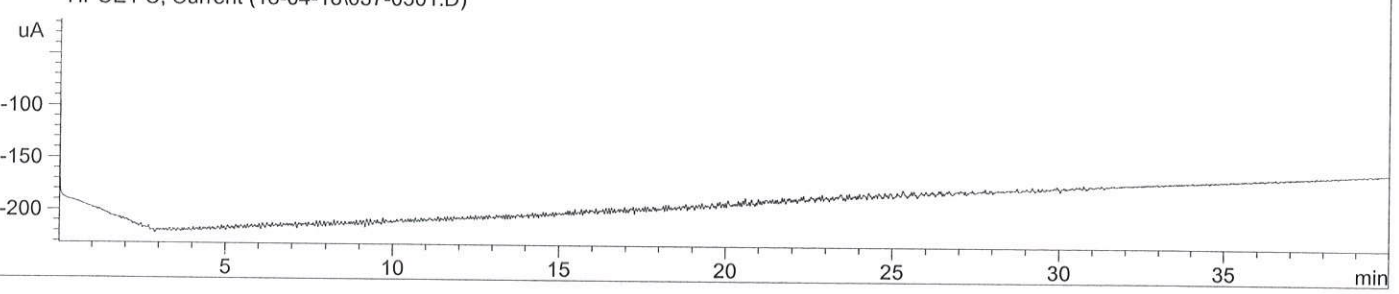
DAD1 D, Sig=280,20 Ref=off (18-04-18\037-0501.D)



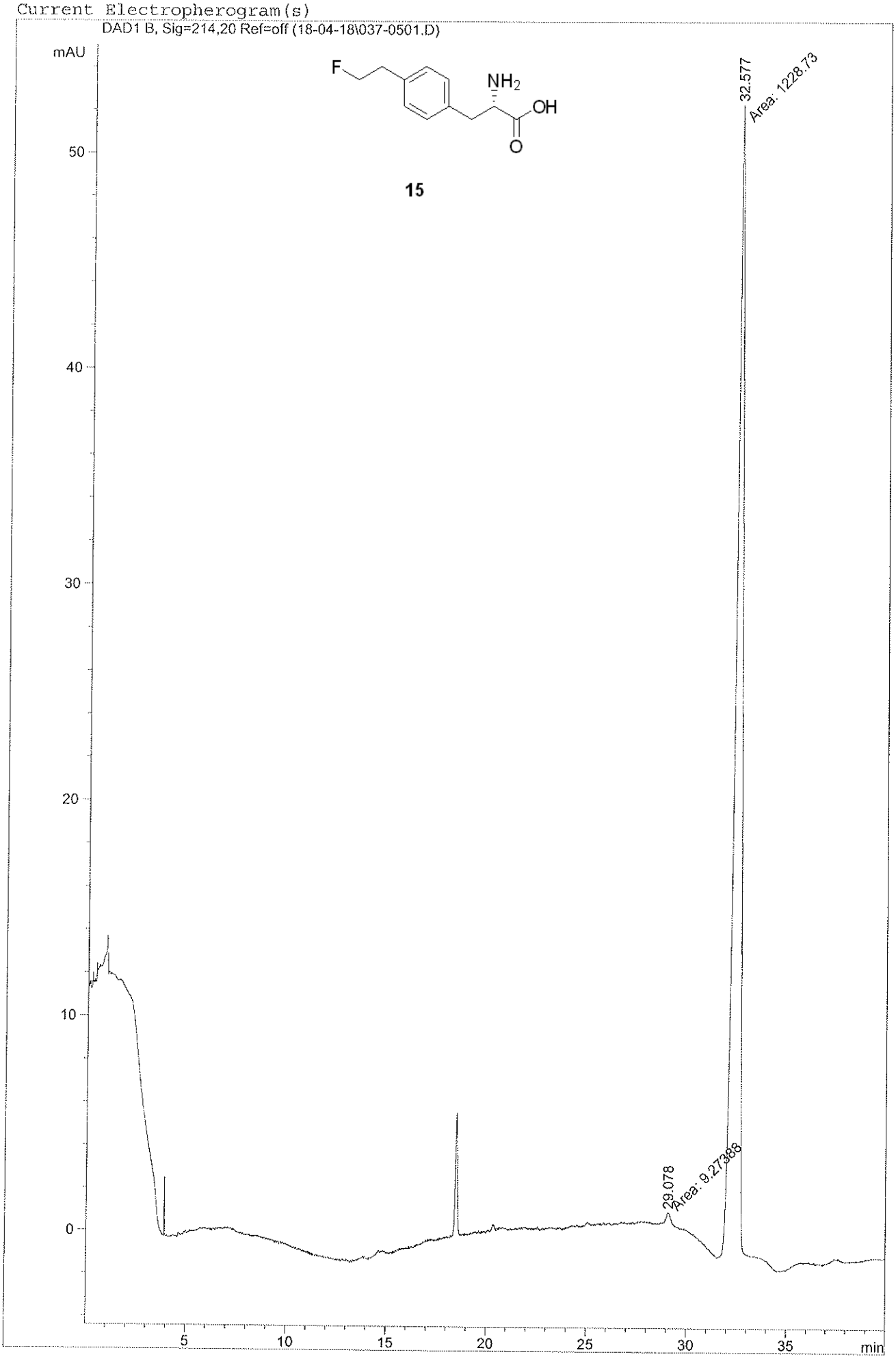
DAD1 E, Sig=310,20 Ref=off (18-04-18\037-0501.D)



HPCE1 C, Current (18-04-18\037-0501.D)

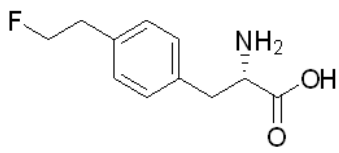


Current Electropherogram(s)



```
==============================================================================
Injection Date  : 4/18/18 10:19:20 PM            Seq. Line :   5
Sample Name     : 19                              Location : Vial 37
Acq. Operator   : Jan Goeman                          Inj :   1
Acq. Method     : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed    : 5/11/11 3:19:59 PM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\CE\DEF_CE.M
Last changed    : 12/15/10 2:53:13 PM by Jan Goeman
Default Method (no analysis)
==============================================================================
                          Area Percent Report
==============================================================================

Sorted By             :      Signal
Area Calculation Mode :      Measured Area
Multiplier            :      1.0000
Dilution              :      1.0000
```



15

```
Signal 1: DAD1 B, Sig=214,20 Ref=off

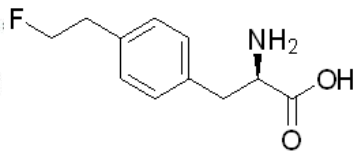
Peak MigTime Type  Width     Area       Height      Area
  #   [min]       [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1  29.078 MM    0.2730    9.27388 5.66264e-1   0.7491
   2  32.577 MM    0.3840 1228.72766   53.33382  99.2509

Totals :                  1238.00154   53.90008

 Results obtained with enhanced integrator! (parabolic interpolation)
==============================================================================
                          *** End of Report ***
```

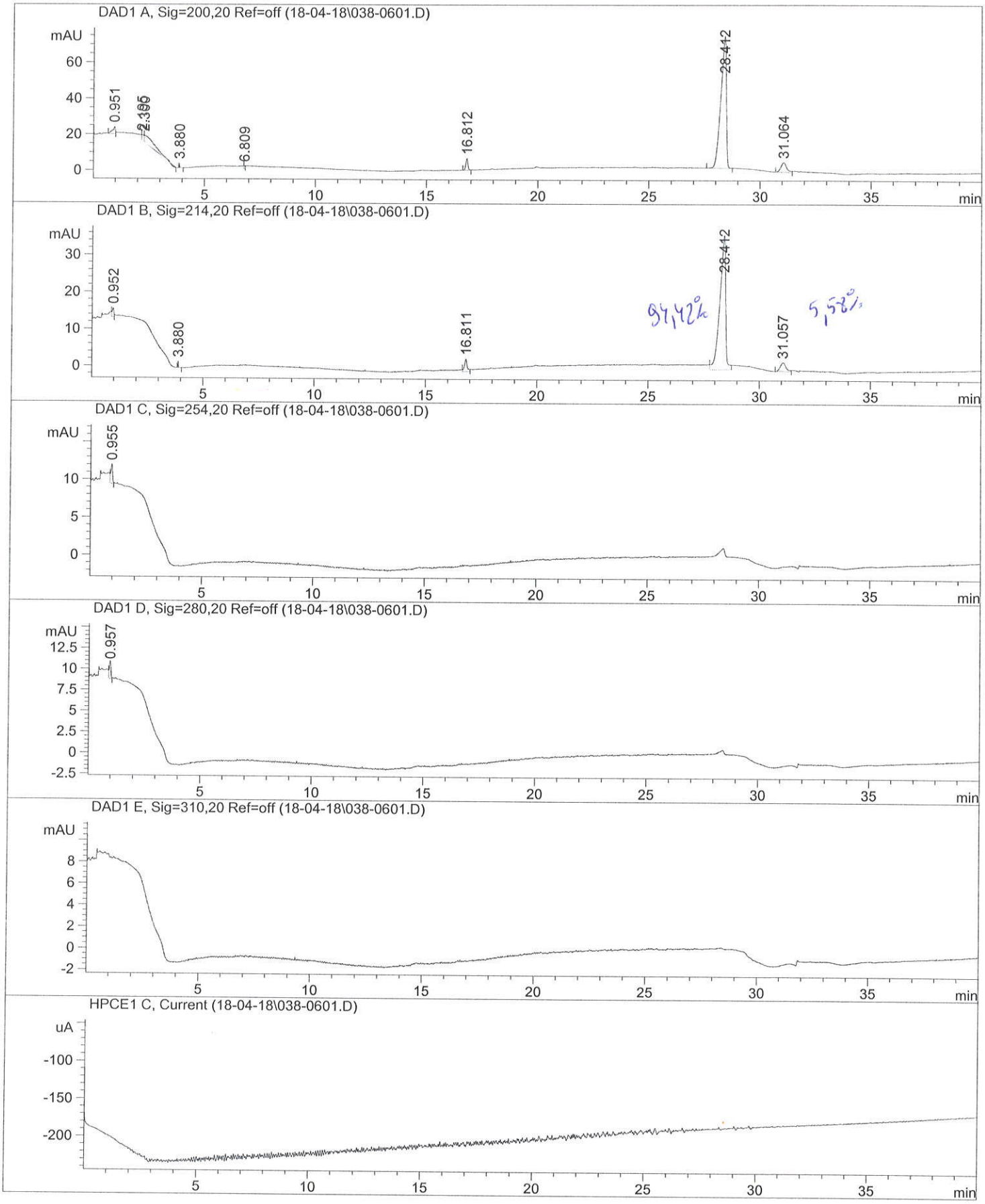
Analysis cpd. 15

```
==========================================================================
Injection Date  : 4/18/18 11:03:41 PM          Seq. Line :   6
Sample Name     : 20                           Location : Vial 38
Acq. Operator   : Jan Goeman                   Inj :   1
Sequence File   : C:\HPCHEM\1\SEQUENCE\TEMP.S
Method          : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed    : 5/11/11 3:19:59 PM by Jan Goeman
CE met buffer 1
L=64,5.cm / d=.50..um / pH.2,5.. .25..mM / SDS. ...mM / .15 y.CD .5...%
==========================================================================
```

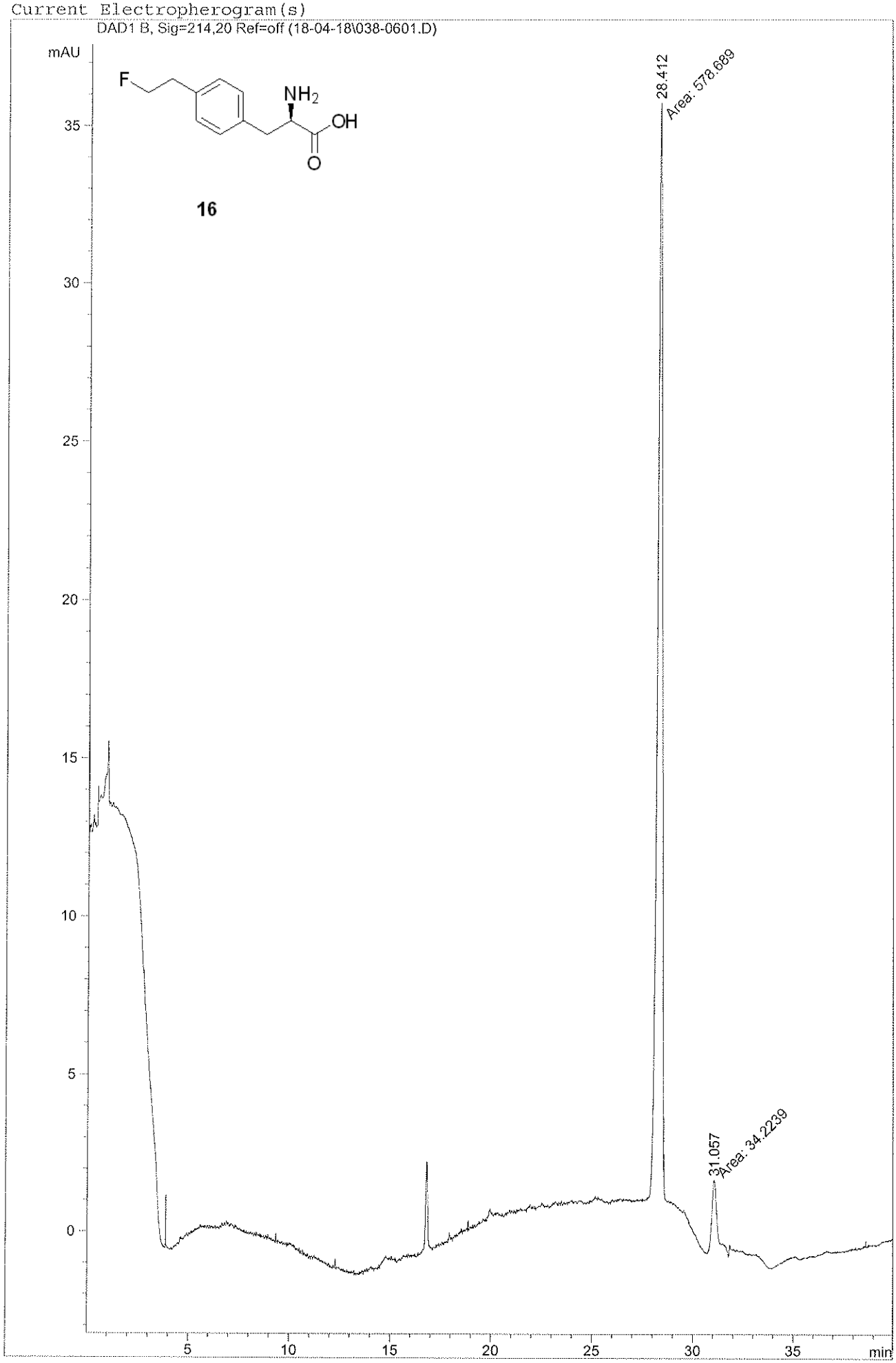


16

Sample: cpd. 16

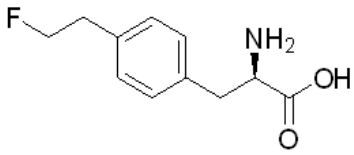


Current Electropherogram(s)



```
==============================================================================
Injection Date  : 4/18/18 11:03:41 PM            Seq. Line :   6
Sample Name     : 20                              Location : Vial 38
Acq. Operator   : Jan Goeman                         Inj :   1
Acq. Method     : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed    : 5/11/11 3:19:59 PM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\CE\DEF_CE.M
Last changed    : 12/15/10 2:53:13 PM by Jan Goeman
Default Method (no analysis)
==============================================================================
                          Area Percent Report
==============================================================================

Sorted By             :      Signal
Area Calculation Mode :      Measured Area
Multiplier            :      1.0000
Dilution              :      1.0000
```



16

Analysis cpd. 16

```
Signal 1: DAD1 B, Sig=214,20 Ref=off

Peak MigTime Type  Width     Area       Height      Area
  #   [min]       [min]    [mAU*s]     [mAU]        %
----|-------|----|--------|----------|----------|--------|
   1  28.412 MM    0.2778  578.68945   34.71428   94.4162
   2  31.057 MM    0.2610   34.22391    2.18517    5.5838

Totals :                   612.91336   36.89945

 Results obtained with enhanced integrator! (parabolic interpolation)
==============================================================================
                          *** End of Report ***
```

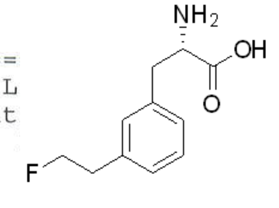
Chiral purity analysis of compound **17** & **18**

Chromatographic conditions for **17** & **18**:

Chiral capillary electrophoresis (CE) employed a 64.5 cm capillary (50 µm diameter). The electrolyte used consisted of 25 mM phosphate buffer pH 2.5 + 5% HS-gammaCD chiral modifier. Analysis time was 40 min at -20 kV.

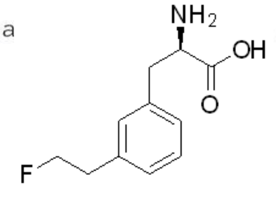
Data File D:\DATA\18-04-18\042-0701.D

Injection Date	: 4/19/18 11:47:57 PM	Seq. L
Sample Name	: 29+31	Locat
Acq. Operator	: Jan Goeman	
Sequence File	: C:\HPCHEM\1\SEQUENCE\TEMP.S	
Method	: C:\HPCHEM\1\METHODS\CE\01-40N20.M	
Last changed	: 5/11/11 3:19:59 PM by Jan Goeman	



17

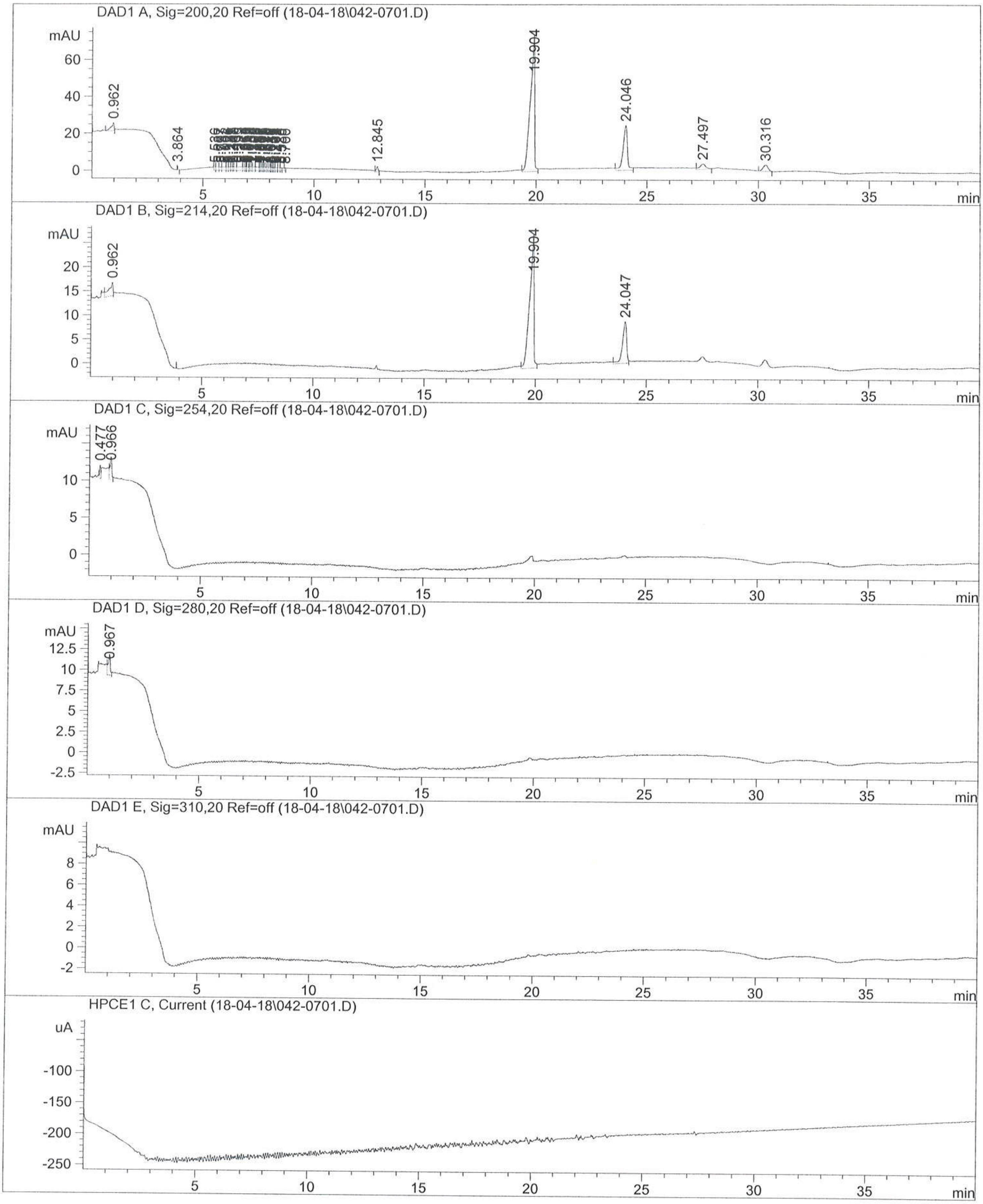
Sa



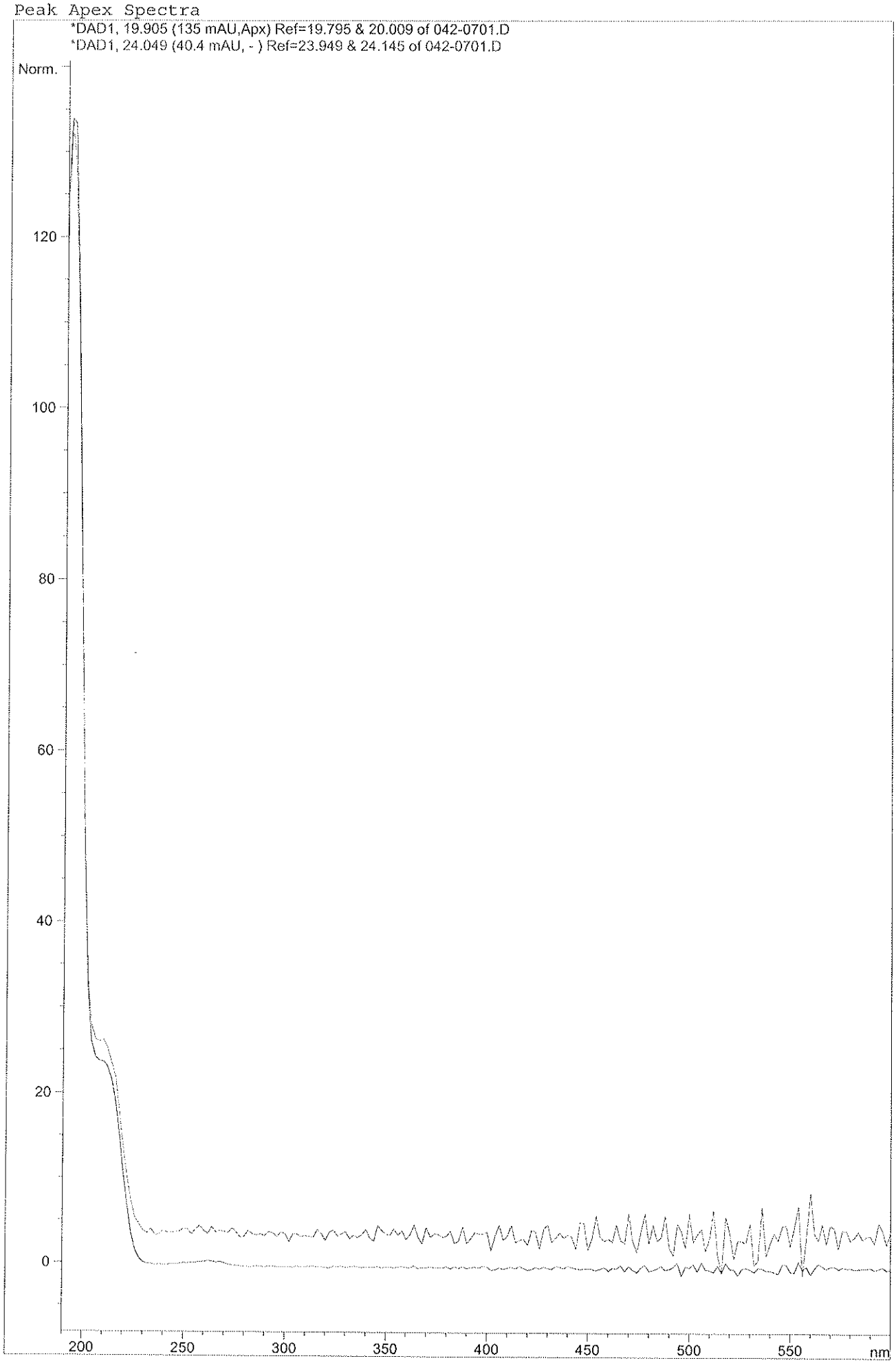
18

CE met buffer 1
L=64,5.cm / d=.50.um / pH.2,5. .25..mM / SDS.-/-...mM / .HS-γCD ..5...%

Optimization of chiral CE
Sample: cpd. 17 & 18

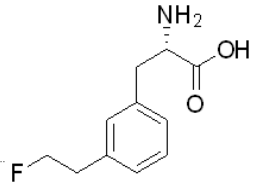


Peak Apex Spectra

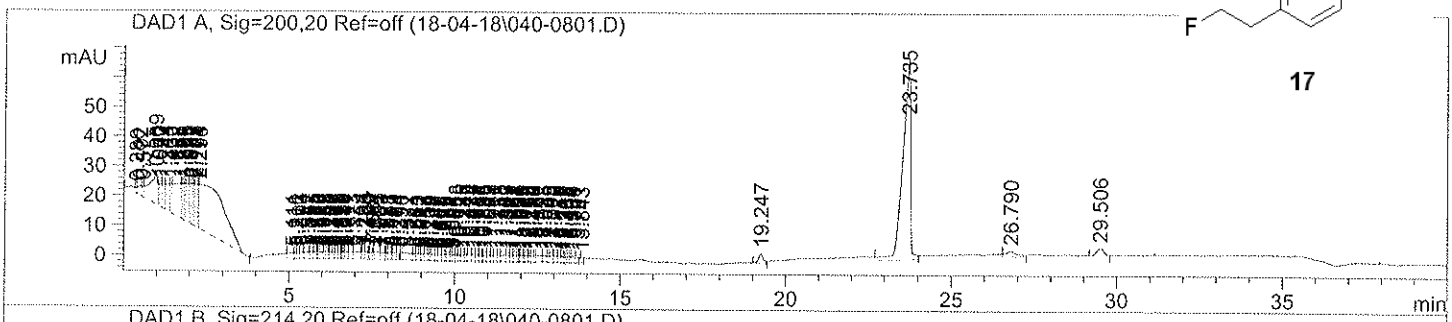


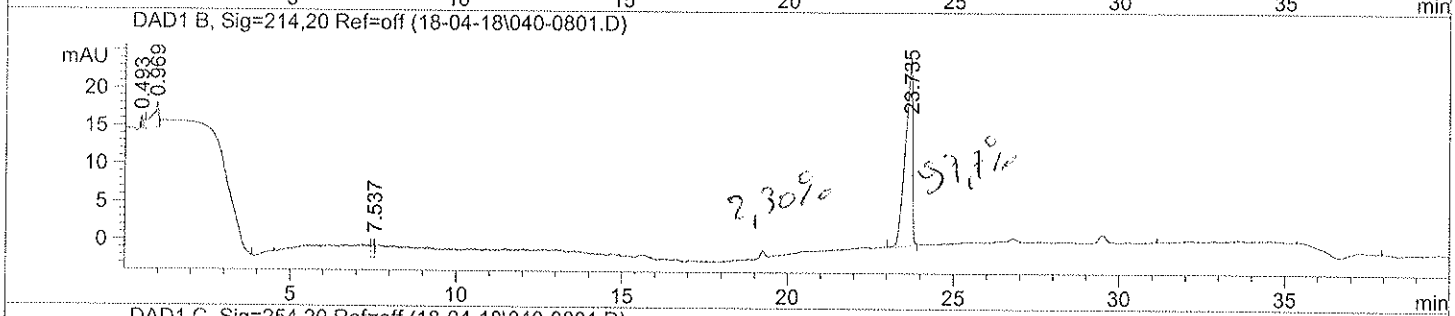
Injection Date : 4/19/18 12:32:19 AM Seq. Line : 8
Sample Name : 29 Location : Vial 40
Acq. Operator : Jan Goeman Inj : 1
Sequence File : C:\HPCHEM\1\SEQUENCE\TEMP.S
Method : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed : 5/11/11 3:19:59 PM by Jan Goeman
CE met buffer 1
L=.64,5.cm / d=.50..um / pH.2,5. .25..mM / SDS ...mM / 115y.CD ..5...%

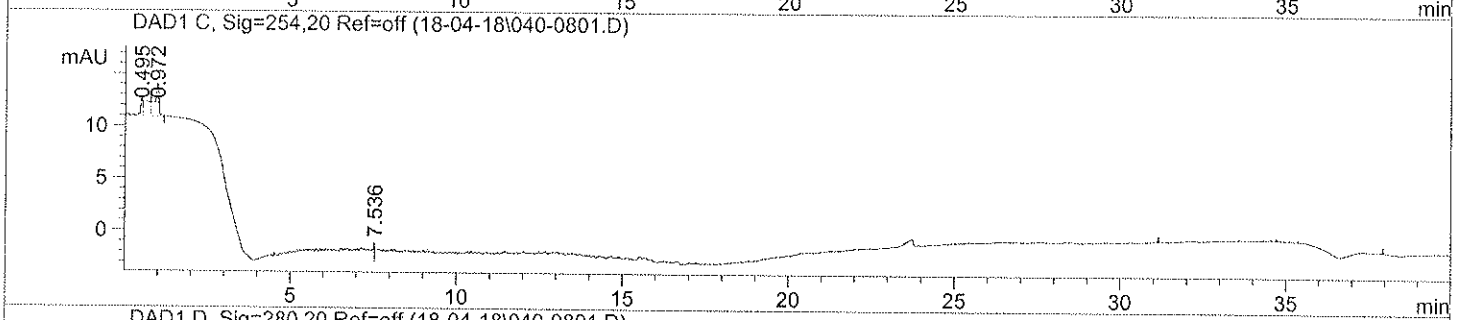
CE analysis
Sample: cpd. 17

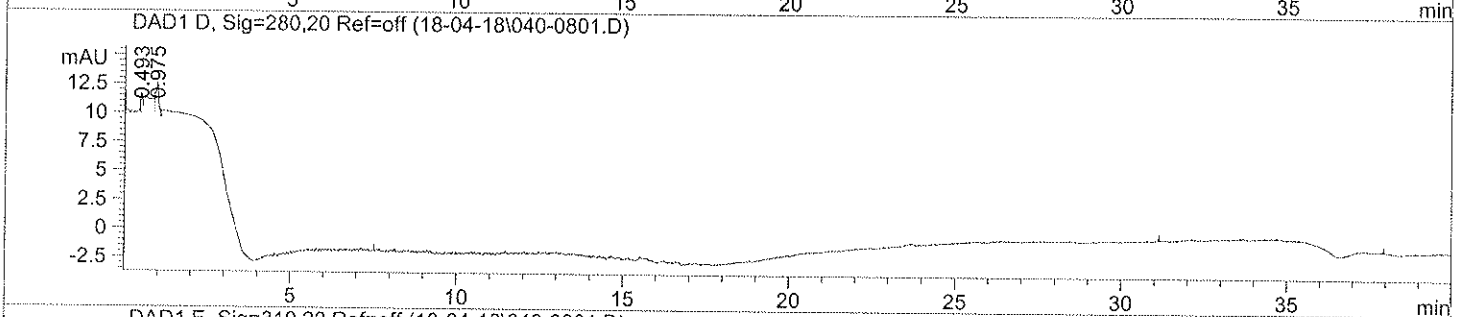


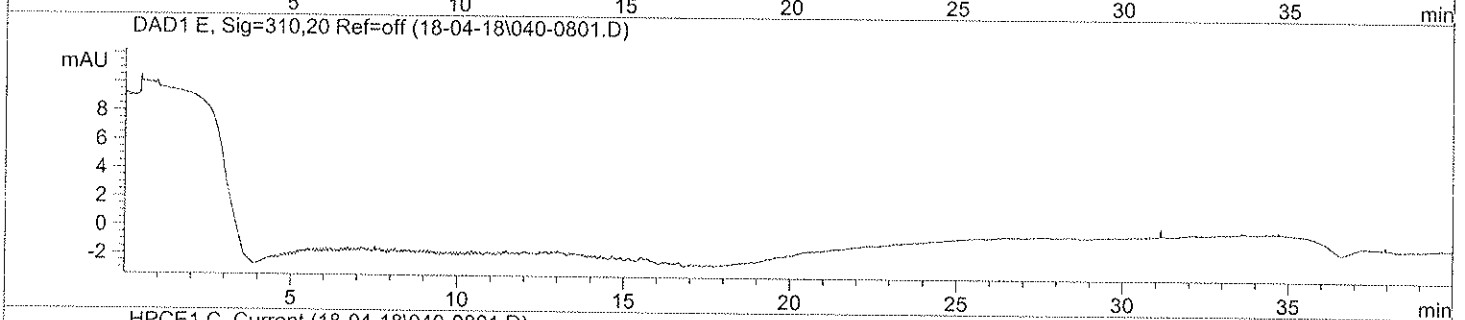
17

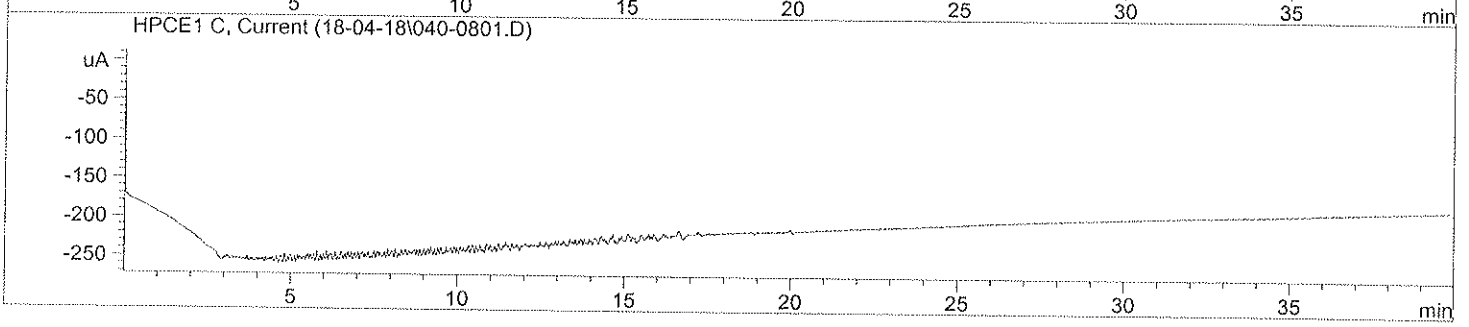




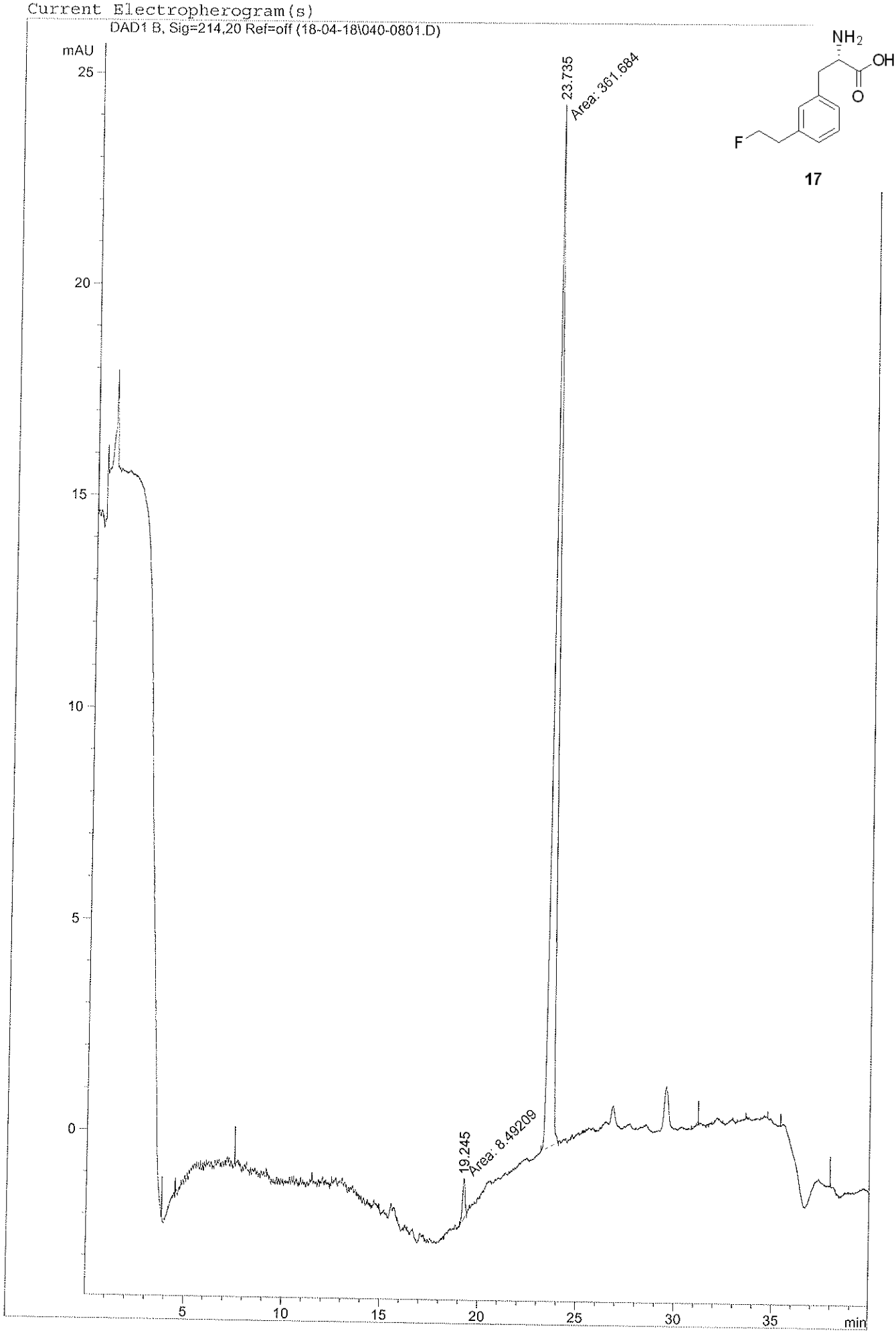








Current Electropherogram(s)



17

```
==============================================================================
Injection Date  : 4/19/18 12:32:19 AM            Seq. Line :   8
Sample Name     : 29                              Location : Vial 40
Acq. Operator   : Jan Goeman                          Inj :   1
Acq. Method     : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed    : 5/11/11 3:19:59 PM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\CE\DEF_CE.M
Last changed    : 12/15/10 2:53:13 PM by Jan Goeman
Default Method (no analysis)
==============================================================================
                          Area Percent Report
==============================================================================

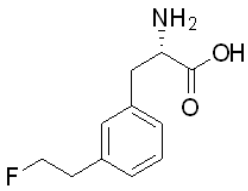
Sorted By             :      Signal
Area Calculation Mode :      Measured Area
Multiplier            :      1.0000
Dilution              :      1.0000


Signal 1: DAD1 B, Sig=214,20 Ref=off

Peak MigTime Type  Width     Area      Height      Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|--------|----|--------|----------|-----------|--------|
   1  19.245 MM    0.1443    8.49209 9.80606e-1   2.2941
   2  23.735 MM    0.2453  361.68427   24.57265  97.7059

Totals :                   370.17636   25.55326

 Results obtained with enhanced integrator! (parabolic interpolation)
==============================================================================
                          *** End of Report ***
```

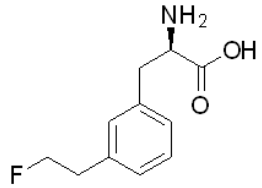


17

Analysis cpd. 17

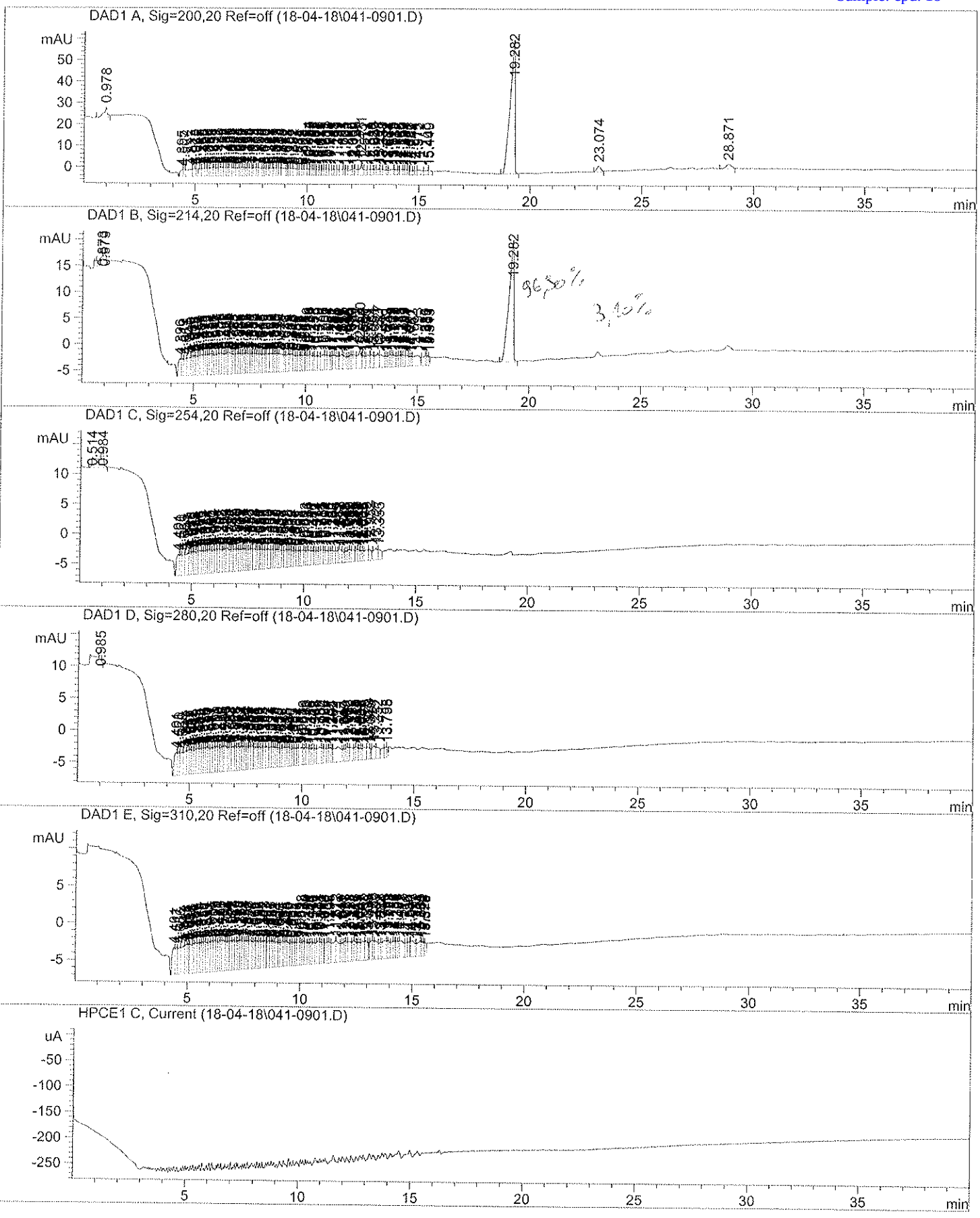
Data File D:\DATA\18-04-18\041-0901.D

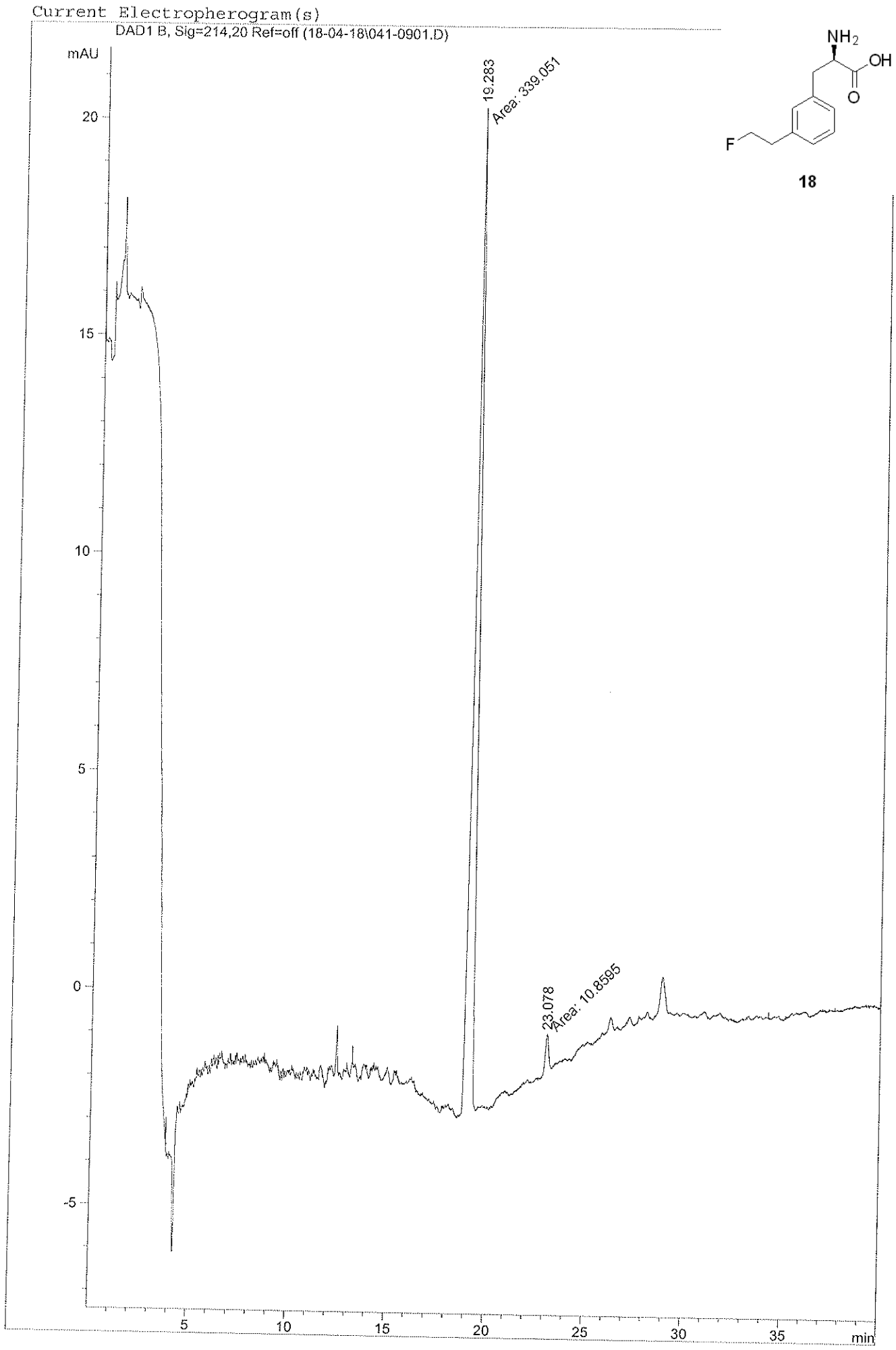
Injection Date : 4/19/18 1:16:34 AM Seq. Line : 9
Sample Name : 30 Location : Vial 41
Acq. Operator : Jan Goeman Inj : 1
Sequence File : C:\HPCHEM\1\SEQUENCE\TEMP.S
Method : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed : 5/11/11 3:19:59 PM by Jan Goeman
CE met buffer 1
L= 64.5 cm / d= 50 um / pH 9.1 25 mM / SDS - mM / HDY CD 5 %



18

Chiral CE analysis
Sample: cpd. 18




Current Electropherogram(s)
DAD1 B, Sig=214,20 Ref=off (18-04-18\041-0901.D)
mAU
20
15
10
5
0
-5
19.283
Area: 339.051
23.078
Area: 10.8595
5
10
15
20
25
30
35
min
NH2
OH
O
F
18

```
==============================================================================
Injection Date  : 4/19/18 1:16:34 AM              Seq. Line :   9
Sample Name     : 30                             Location : Vial 41
Acq. Operator   : Jan Goeman                        Inj :   1
Acq. Method     : C:\HPCHEM\1\METHODS\CE\01-40N20.M
Last changed    : 5/11/11 3:19:59 PM by Jan Goeman
Analysis Method : C:\HPCHEM\1\METHODS\CE\DEF_CE.M
Last changed    : 12/15/10 2:53:13 PM by Jan Goeman
Default Method (no analysis)
==============================================================================
                          Area Percent Report
==============================================================================

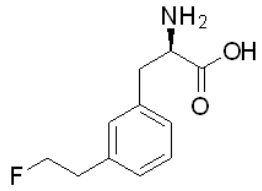
Sorted By             :      Signal
Area Calculation Mode :      Measured Area
Multiplier            :      1.0000
Dilution              :      1.0000


Signal 1: DAD1 B, Sig=214,20 Ref=off

Peak MigTime Type  Width     Area       Height      Area
  #   [min]       [min]    [mAU*s]     [mAU]        %
----|--------|----|-------|----------|----------|--------|
   1  19.283 MM    0.2450  339.05106   23.06212  96.8965
   2  23.078 MM    0.1991   10.85953 9.08849e-1   3.1035

Totals :                   349.91059   23.97097

 Results obtained with enhanced integrator! (parabolic interpolation)
==============================================================================
                          *** End of Report ***
```



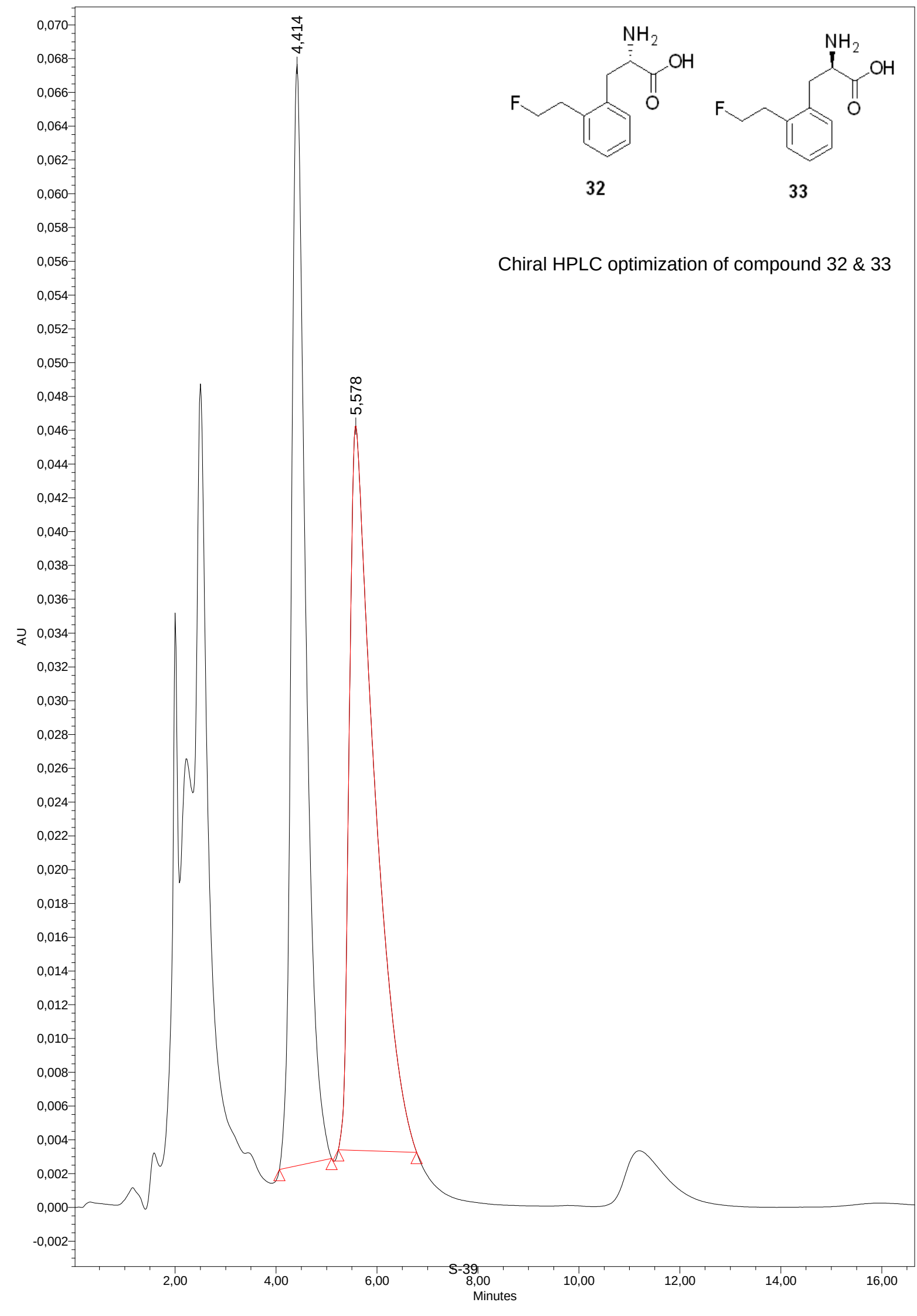
18

Sample: cpd. 18

Chiral purity analysis of compound **32** & **33**

Chromatographic conditions for **32** & **33**:

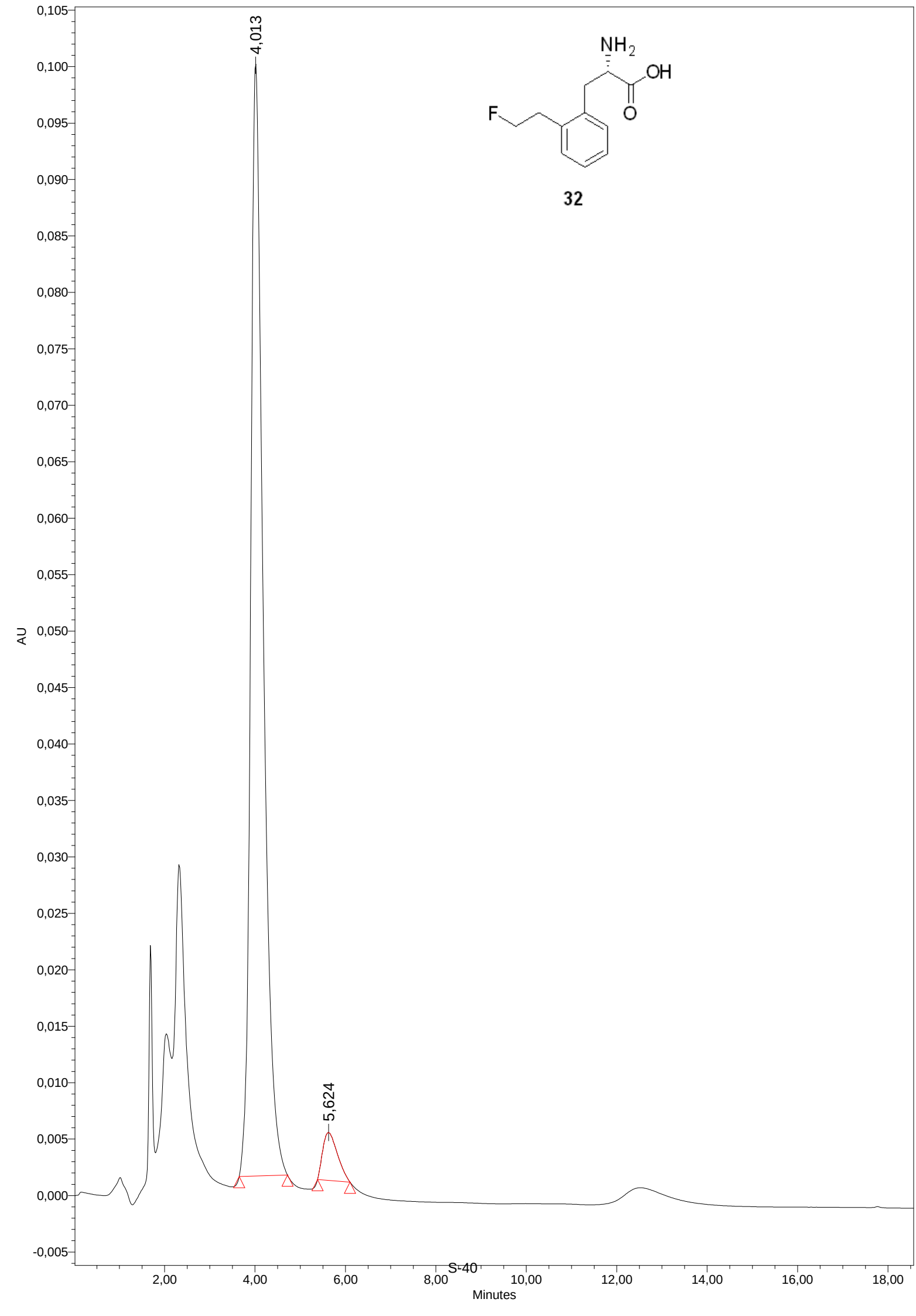
Astec® Chirobiotic-T column, 125 x 4.6 mm, particle size 5 µm. Column temperature was regulated at ambient temperature. The chromatographic run was performed isocratically with a mixture of EtOH/water (8/2) at a flow rate of 1 mL/min.



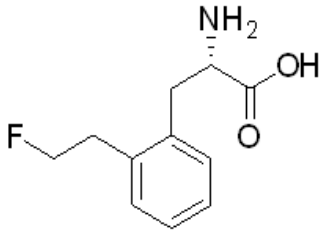
32

33

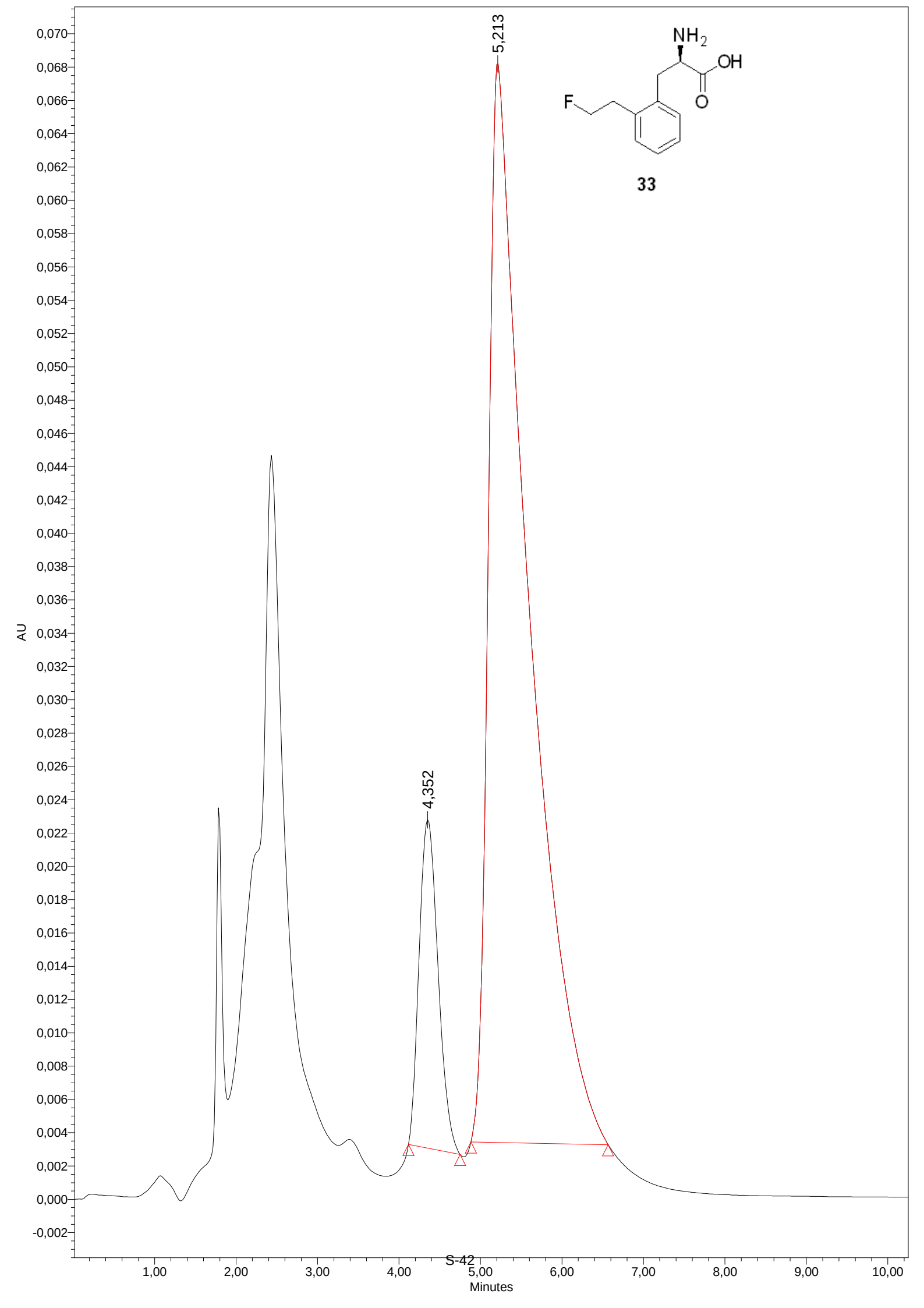
Chiral HPLC optimization of compound 32 & 33


4,013
5,624
NH2
OH
O
F
32
AU
0,105
0,100
0,095
0,090
0,085
0,080
0,075
0,070
0,065
0,060
0,055
0,050
0,045
0,040
0,035
0,030
0,025
0,020
0,015
0,010
0,005
0,000
-0,005
2,00
4,00
6,00
8,00
10,00
12,00
14,00
16,00
18,00
Minutes
S-40

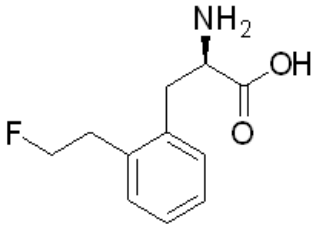
	Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Amount	Units	Peak Type	Peak Codes
1		4,013	1896688	95,21	98536	bb			Unknown	
2		5,624	95519	4,79	4250	bb			Unknown	



32

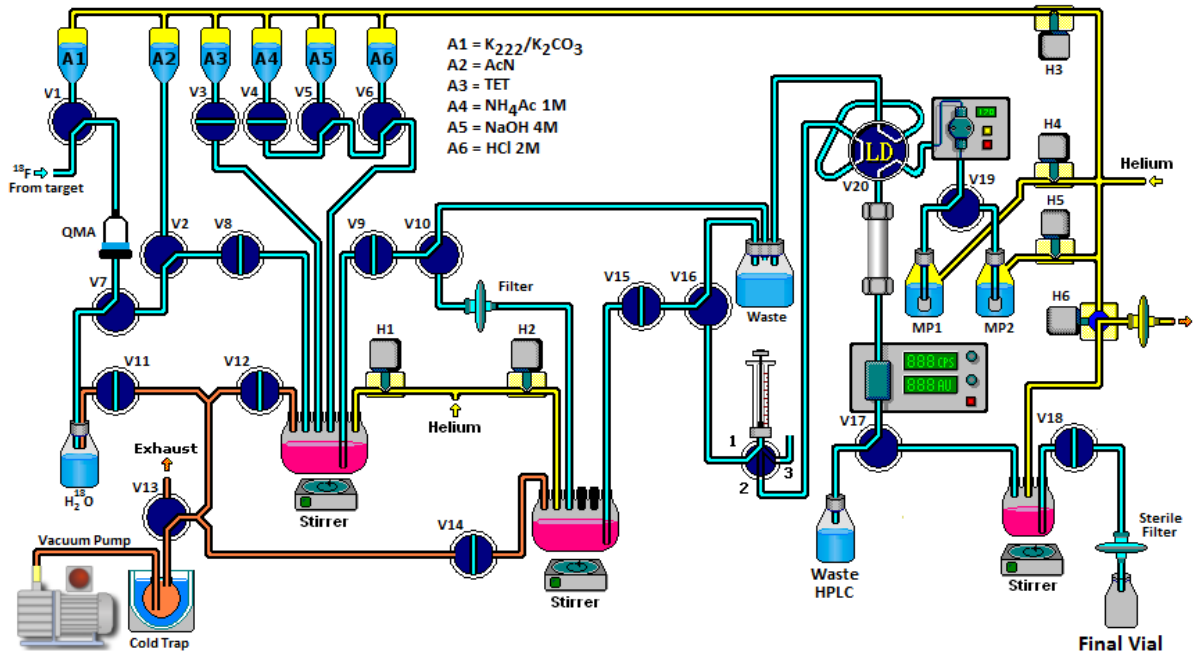

5,213
4,352
NH2
OH
O
F
33
AU
0,070
0,068
0,066
0,064
0,062
0,060
0,058
0,056
0,054
0,052
0,050
0,048
0,046
0,044
0,042
0,040
0,038
0,036
0,034
0,032
0,030
0,028
0,026
0,024
0,022
0,020
0,018
0,016
0,014
0,012
0,010
0,008
0,006
0,004
0,002
0,000
-0,002
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
9,00
10,00
Minutes
S-42

	Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Amount	Units	Peak Type	Peak Codes
1		4,352	319629	12,38	19742	bb			Unknown	
2		5,213	2262906	87,62	64814	bb			Unknown	



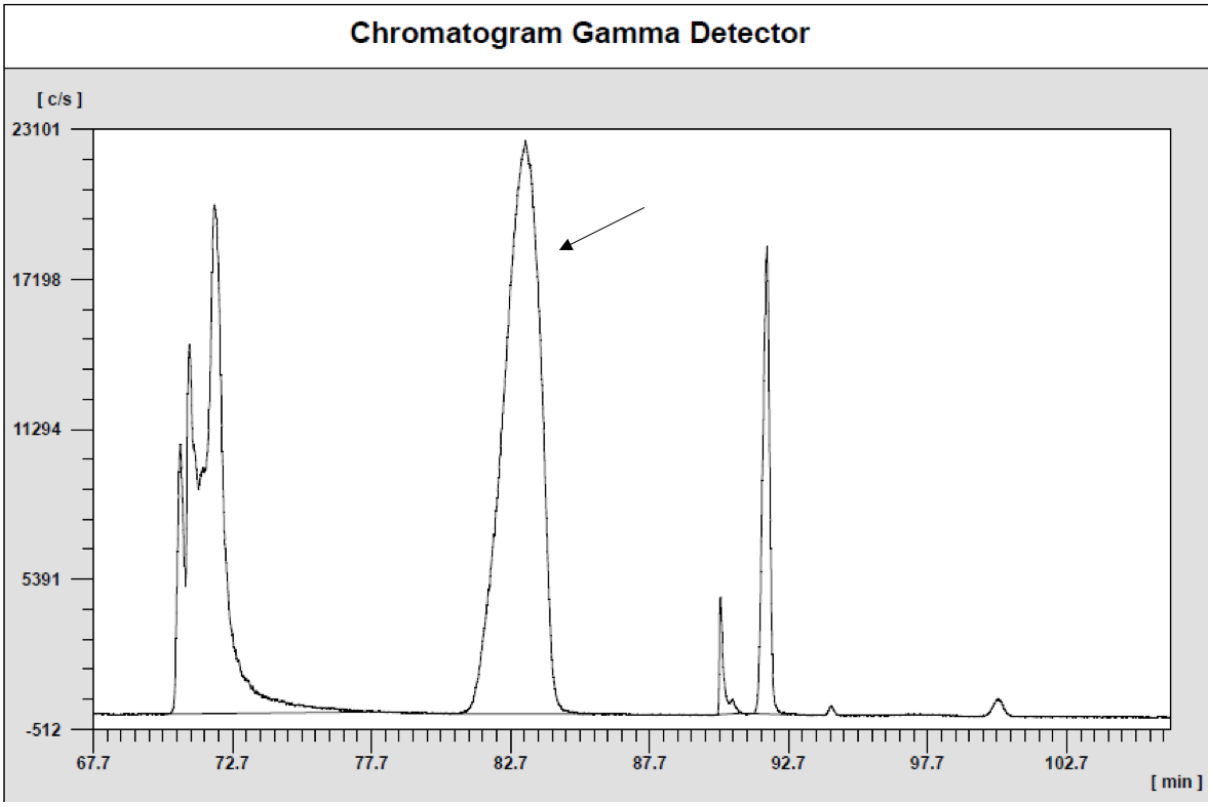
33

Radiosynthesis of [^{18}F]-32 (2-[^{18}F]-FELP): Flow scheme



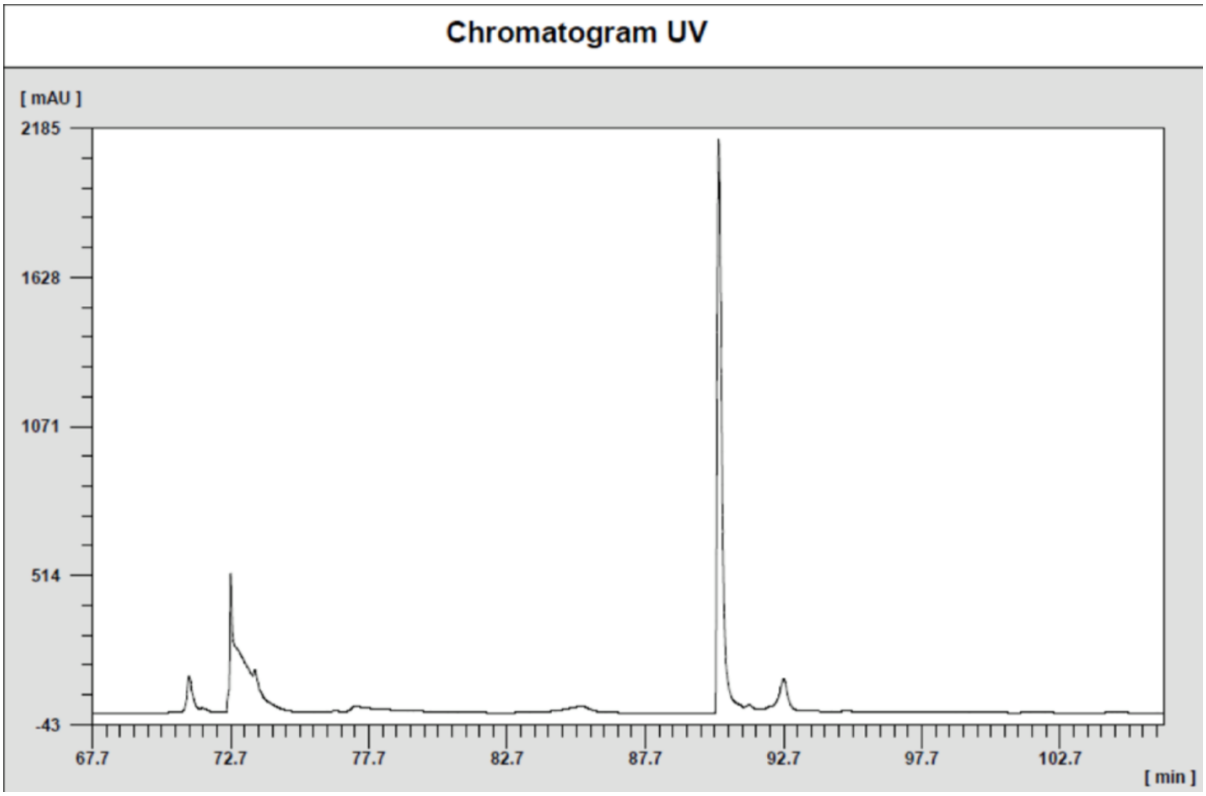
Supplementary Figure 1: Flow scheme of custom Synthra RN plus module (Synthra GmbH, Hamburg,Germany).

Radiogram of [^{18}F]-**32** (2-[^{18}F]-FELP)



Supplementary Figure 2: Radiogram of radiosynthesized **2-[^{18}F]FELP**. The arrow indicates the **2-[^{18}F]FELP** peak**.**

UV Chromatogram of [^{18}F]-**32** (2-[^{18}F]-FELP)

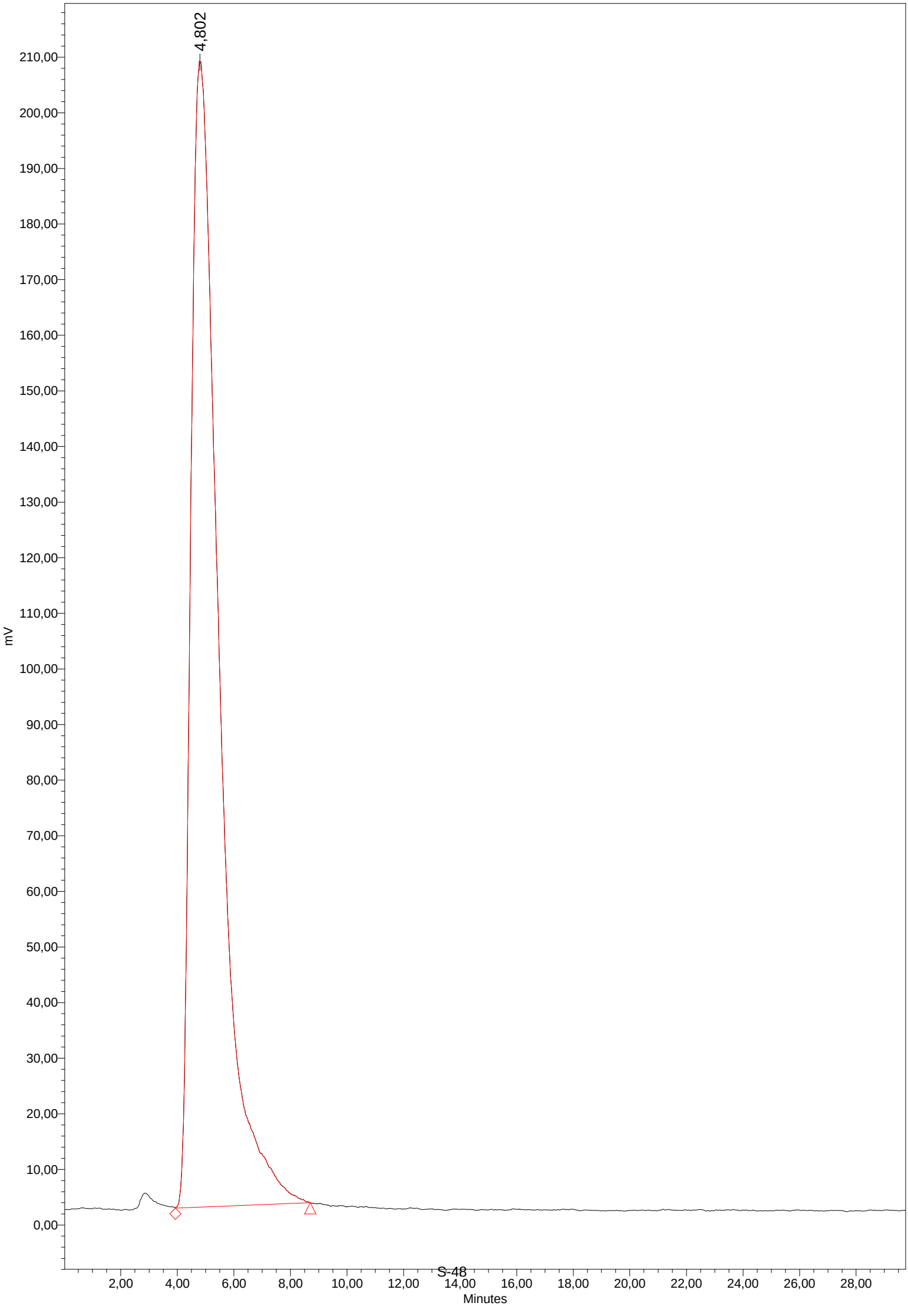


Supplementary Figure 3: UV chromatogram (254 nm) of radiosynthesized **2-[^{18}F]FELP**.

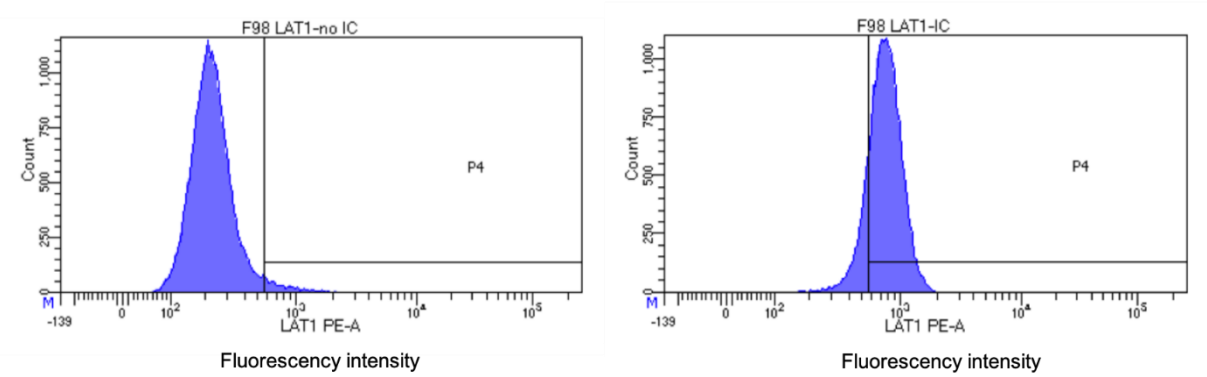
Chiral purity analysis of compound [^{18}F]-**32** (2-[^{18}F]-FELP)

Chromatographic conditions for 2-[^{18}F]-**32**:

Astec® Chirobiotic-T column, 125 x 4.6 mm, particle size 5 µm. Column temperature was regulated at ambient temperature. The chromatographic run was performed isocratically with a mixture of EtOH/water (8/2) at a flow rate of 1 mL/min.

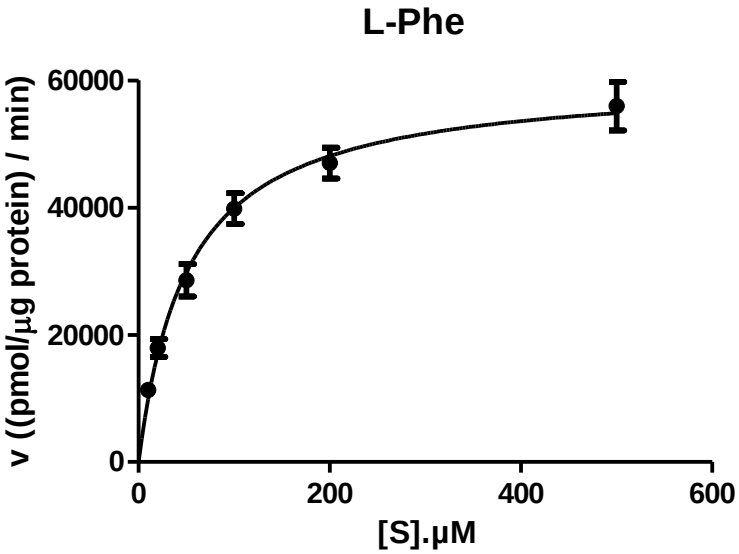

4,802
mV
210,00
200,00
190,00
180,00
170,00
160,00
150,00
140,00
130,00
120,00
110,00
100,00
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
2,00
4,00
6,00
8,00
10,00
12,00
14,00
16,00
18,00
20,00
22,00
24,00
26,00
28,00
Minutes

Flow cytometry



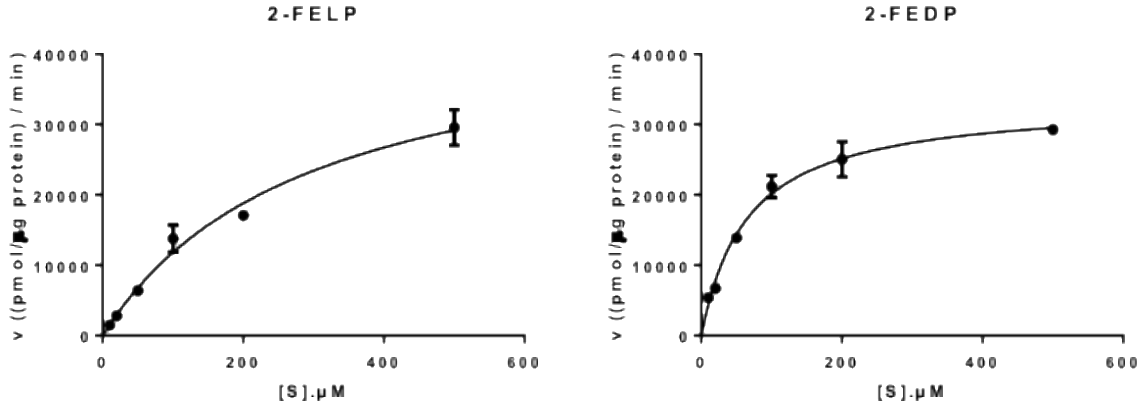
Supplementary Figure 4: Flow cytometry: LAT-1 expression of F98 cells. Left histogram represents surface staining (negative control). Right histogram represents intracellular staining.

Michaelis-Menten plots



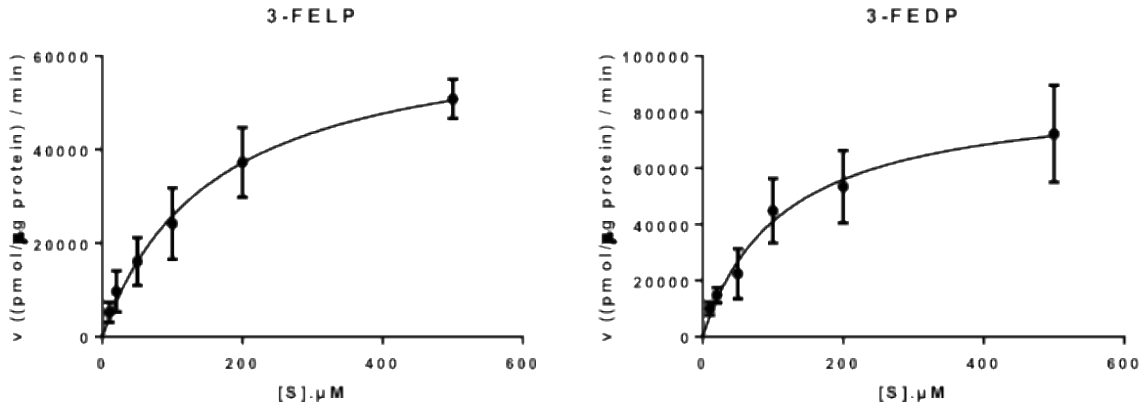
Supplementary Figure 5: Michaelis-Menten plot of [^{3}H]-L-Phe uptake.

Incubation time = 1 min. (*n*=6) K_m = 51.53 ± 7.80 µM



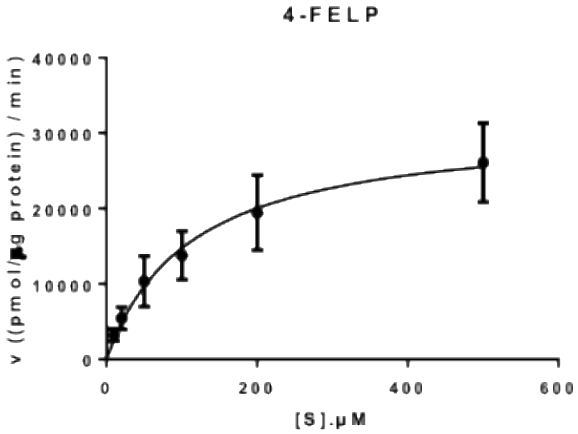
Supplementary Figure 6: Michaelis-Menten plot of [^{3}H]-L-Phe uptake in the presence of **2-FELP** (**32**) (left) or **2-FEDP** (**33**) (right). Incubation time = 1 min. (*n*=6)

K_i **2-FELP** (**32**) = 16.09 ± 3.74 µM. K_i **2-FEDP** (**33**) = 359.4 ± 47.1 µM.

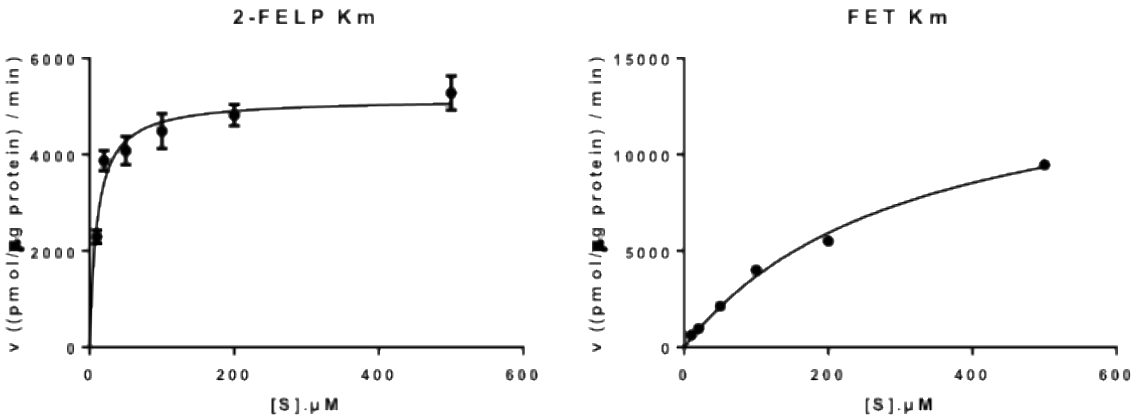


Supplementary Figure 7: Michaelis-Menten plot of [^{3}H]-L-Phe uptake in the presence of **3-FELP** (**17**) (left) or **3-FEDP** (**18**) (right). Incubation time = 1 min. (*n*=6)

K_i **3-FELP** (**17**) = 48.95 ± 4.92 µM. K_i **3-FEDP** (**18**) = 83.75 ± 28.1 µM

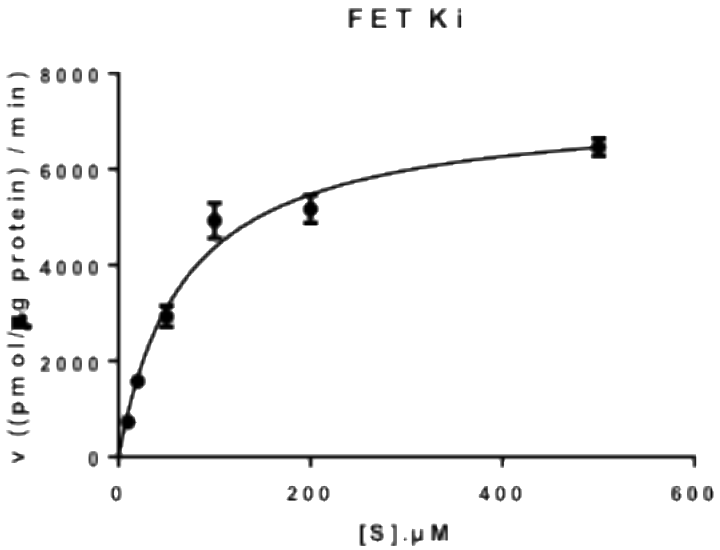


Supplementary Figure 8: Michaelis-Menten plot of [^{3}H]-L-Phe uptake in the presence of **4-FELP** (**15**). Incubation time = 1 min. (*n*=6) K_i **4-FELP** (**15**) = 85.55 ± 4.40 µM.



Supplementary Figure 9: Michaelis-Menten plot **2-[^{18}F]FELP (32)** (left) and **[^{18}F]FET** (right) uptake. Incubation time = 1 min. (*n* = 6)

K_m **2-[^{18}F]FELP (32)** = 12.54 ± 3.82 µM. K_m **[^{18}F]FET** = 312.9 ± 25.6 µM



Supplementary Figure 10: Michaelis-Menten plot of [^{3}H]-L-Phe uptake in the presence of **FET**. Incubation time = 1 min. (*n* = 6) K_i **FET** = 307.2 ± 4.29 µM