Supporting Information

Discovery, Radiolabeling, and Evaluation of Subtype-Selective Inhibitors for Positron Emission Tomography Imaging of Brain Phosphodiesterase-4D

Yuichi Wakabayashi,[1*] Sanjay Telu[1*], Rachel M. Dick[1], Masahiro Fujita[1], Maarten Ooms[1], Cheryl L. Morse[1], Jeih-San Liow[1], Jinsoo S Hong[1], Robert L. Gladding,[1] Lester S. Manly[1], Sami S. Zoghbi[1], Xuesheng Mo[2], Emily C. D'Amato[2], Janice A. Sindac[2], Richard A. Nugent[2], Brian E. Marron[2], Mark E. Gurney[2§], Robert B. Innis[1], Victor W. Pike[1§]

[1]National Institute of Mental Health, Bethesda, MD, USA

[2]Tetra Therapeutics, Grand Rapids, MI, USA

- Equal authorship priority

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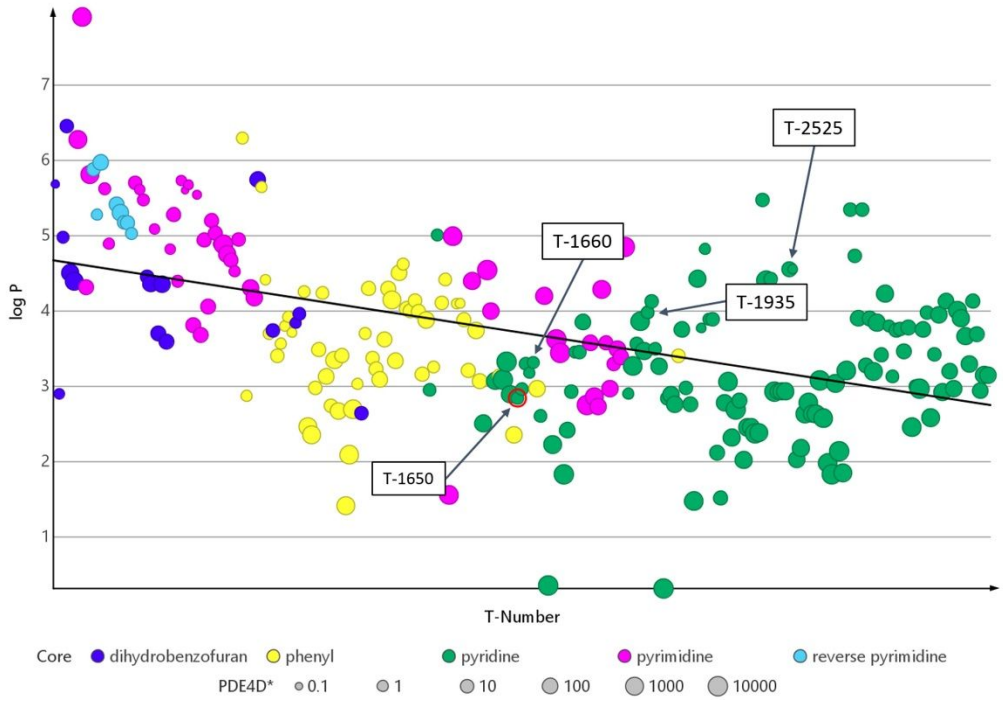
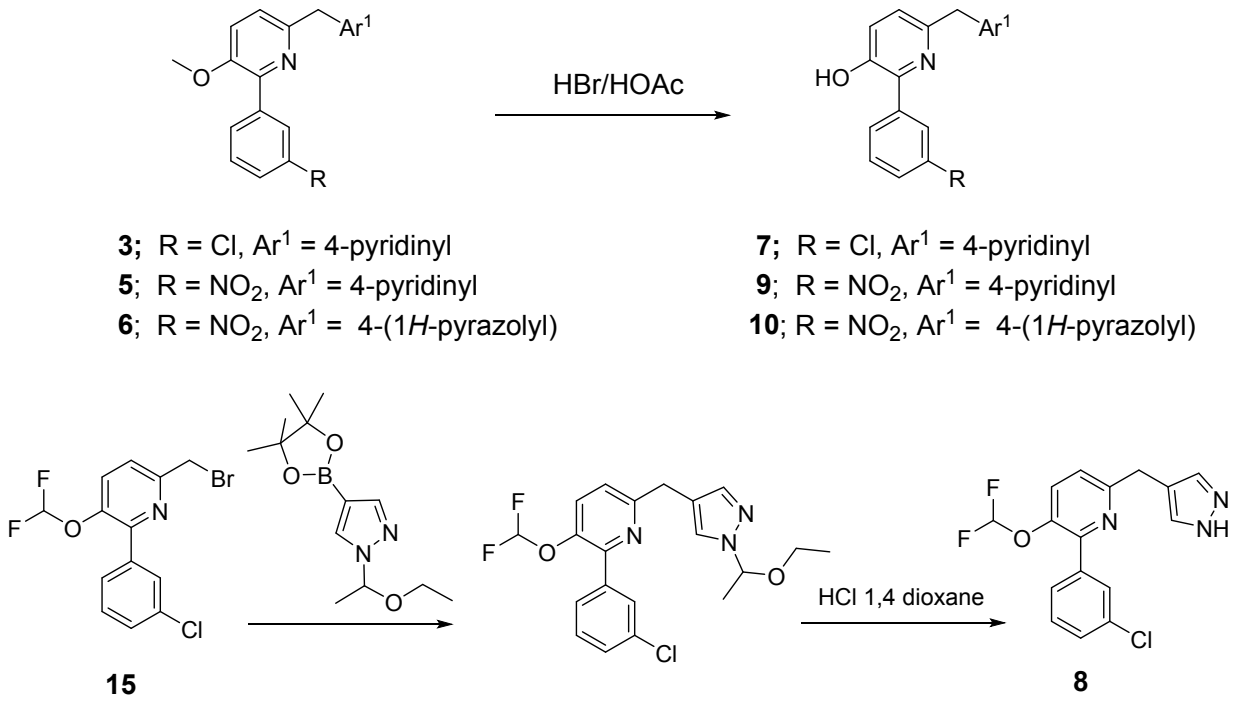


Figure S1. Time-sequence for reduction in clog*P* across the different chemical series. Compounds evaluated in monkey PET studies are named. The PDE4D inhibitory potency is shown by the size of the symbol.

Syntheses of desmethyl precursors 7–10

(See Appendix 1 for characterization data: HPLC analyses, mass, and ^{1}H- and ^{13}C-NMR spectra).

Scheme S1. Synthesis of desmethyl precursors



Preparation of 2-(3-chlorophenyl)-3-hydroxy-6-[(pyridin-4-yl)methyl]pyridine (7; T2009). Compound **3 (**T1953; 55 mg, 0.18 mmol) was dissolved in AcOH (2 mL) and treated with 48% HBr (3 mL). The vial was heated to 120 ℃ for 48 h. The reaction mixture was condensed and then neutralized carefully with $NaHCO_3$ solution. The product was extracted 3 times with ethyl acaetate, dried with Na_2SO_4, and concentrated, and then purified by silica gel chromatography (EtOAc/DCM, 1:1) to give **7** as a white solid: 44 mg (0.15 mmol, 82%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.2 (s, 1H), 8.47 (d, J = 5.6 Hz, 2H), 8.05 (m, 3H), 7.96 (m, 1H), 7.44 (m, 2H), 7.31 (m, 3H), 7.17 (d, J = 8.3 Hz, 2H), 4.08 (s, 2H); TOF MS ES$^+$ *m/z* 297.0 $[M+H]^+$; HPLC Method A, t_R = 2.23 min.

Preparation of 3-hydroxy-2-(3-nitrophenyl)-6-[(pyridin-4-yl)methyl]pyridine (9; T1842). Using the method of compound **7** and purification by silica gel chromatography (5% MeOH/DCM) gave **9** as a white solid: (56%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.51 (s, 1H), 8.93 (t, J = 1.9 Hz, 1H), 8.54 (d, J = 8.0 Hz, 1H), 8.47 (d, J = 5.9 Hz, 1H), 8.22 (m, 1 H), 7.74 (t, J = 8.1 Hz, 1H), 7.34 (m, 3H), 7.23 (d, J = 8.0 Hz, 1H), 4.11 (s, 2H); TOF MS ES$^+$ *m/z* 308.1 $[M+H]^+$; HPLC Method A, t_R = 2.25 min.

Preparation of 2-(3-nitrophenyl)-3-hydroxy-6-[(1*H*-pyrazol-4-yl)methyl]pyridin-3-ol (10; T1720). Using the method of compound **7** and purification by silica gel chromatography (3–10% MeOH/DCM) gave **10** as a white solid: (45%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.59 (s, 1H),

10.37 (s, 1H), 8.96 (t, J = 1.9 Hz, 1H), 8.57 (dt, J = 8.0, 1.2 Hz, 1H), 8.22 (ddd, 8.2 Hz, 2.3 Hz, 1.0 Hz, 1 H), 7.75 (t, J = 8.1 Hz, 1H), 7.55 (br s, 2H), 7.34 (d, J = 8.3 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1 H), 3.92 (s, 2H); TOF MS ES^+ *m/z* 296.9 $[M+H]^+$; HPLC Method B, t_R = 2.37 min.

Preparation of 2-(3-chlorophenyl)-3-(difluoromethoxy)-6-[(1*H*-pyrazol-4-yl)methyl]pyridine (8; T2517)

*2-(3-Chlorophenyl)-3-(difluoromethoxy)-6-[(1-ethoxyethyl-1*H*-pyrazol-4-yl)methyl]pyridine*. In a 25-mL round bottom flask, 6-(bromomethyl)-2-(3-chlorophenyl)-3-difluoromethoxypyridine (**15**; 490 mg, 1.41 mmol) was dissolved in 1,4-dioxane:water (4:1, 6 mL) and then treated with 1-(1-ethoxyethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (750 mg, 2.82 mmol), K_3PO_4 (600 mg, 2.83 mmol), and Pd(dppf)Cl_2 (110 mg, 0.15 mmol). Argon was bubbled through the solution for 5 min, then the flask was sealed under argon and heated at 90 ℃ for 12 h. The reaction mixture was concentrated under reduced pressure and the residue partitioned between ethyl acetate and water. The layers were separated, and the organic phase dried with Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography using 100% DCM to give the product as a white solid: 150 mg (0.39 mmol, 28%).

*2-(3-Chlorophenyl)-3-(difluoromethoxy)-6-[(1*H*-pyrazol-4-yl)methyl]pyridine (**8**; T2517):* To a solution of 2-(3-chlorophenyl)-3-(difluoromethoxy)-6-[(1-ethoxyethyl-1*H*-pyrazol-4-yl)methyl]pyridine (140 mg, 0.37 mmol) in MeOH (2 mL) was added water (50 μL) and 4 M HCl in 1,4-dioxane (200 μL). After stirring the mixture at room temperature for 18 h, the solvent was removed and the residue chromatographed on silica gel using 2% EtOAc/DCM to give **8**: 110 mg (0.32 mmol, 87%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.89 (m, 1H), 7.78 (m, 1H), 7.54 (m, 3H), 7.42 (m, 3H), 7.14 (d, J = 8.4 Hz, 1H), 6.49 (t, J = 72 Hz, 1H), 4.13 (s, 2H); TOF MS ES^+ *m/z* 336.2 $[M+H]^+$, 377.1 $[M+ACN+H]^+$; HPLC Method A, t_R = 3.41 min.

Table S1: Radioligand HPLC purification conditions.

Radioligand	Column[a]	Mobile phase (v/v)	t_R (min)[b]
[^{11}C]**3** ([^{11}C]T1953)	Luna C18(2),10 µm	MeCN-H_2O (62/38)	9.4
[^{11}C]**4** ([^{11}C]T2525)	Luna C18(2),10 µm	MeCN-0.1 M aq. HCO_2NH_4 (55/45)	11.8
[^{11}C]**5** ([^{11}C]T1660)	Luna C18(2),10 µm	MeCN-H_2O (60/40)	8.0
[^{11}C]**6** ([^{11}C]T1650)	Luna C18, 10 µm	MeCN-0.1 M aq. HCO_2NH_4 (45/55)	9.9
[^{11}C]**6** ([^{11}C]T1650)[c]	Luna C18, 5 µm	MeCN-0.1 M aq. HCO_2NH_4 (45/55)	10.6

[a] All HPLC columns had dimensions of 10 mm o.d. × 250 mm and were eluted at 6 mL/min.
[b] Carrier for each radioligand elutes slightly earlier because UV absorbance and radioactivity detectors are in series.
[c] For human productions.

Table S2: Radioligand HPLC analysis conditions.

Radioligand	Column[a]	Mobile phase (v/v)	t_R (min)[b]
[^{11}C]**3** ([^{11}C]T1953)	Luna C18, 10 µm	MeCN-H_2O (60/40)	7.9
[^{11}C]**4** ([^{11}C]T2525)	Luna C18, 10 µm	MeCN-0.1 M aq. HCO_2NH_4 (60/40)	5.1
[^{11}C]**5** ([^{11}C]T1660)	Luna C18, 10 µm	MeCN-0.1 M aq. HCO_2NH_4 (60/40)	5.4
[^{11}C]**6** ([^{11}C]T1650)	Luna C18(2), 10 µm	MeCN-0.1 M aq. HCO_2NH_4 (50/50)	4.0

[a] All the HPLC columns had dimensions of 4.6 mm o.d. × 250 mm and were eluted at 2.0 mL/min.
[b] Carrier for each radioligand elutes slightly earlier because UV absorbance and radioactivity detectors are in series.

All the formulated radioligands were chemically and radiochemically stable for at least 1 h by radio-HPLC analysis.

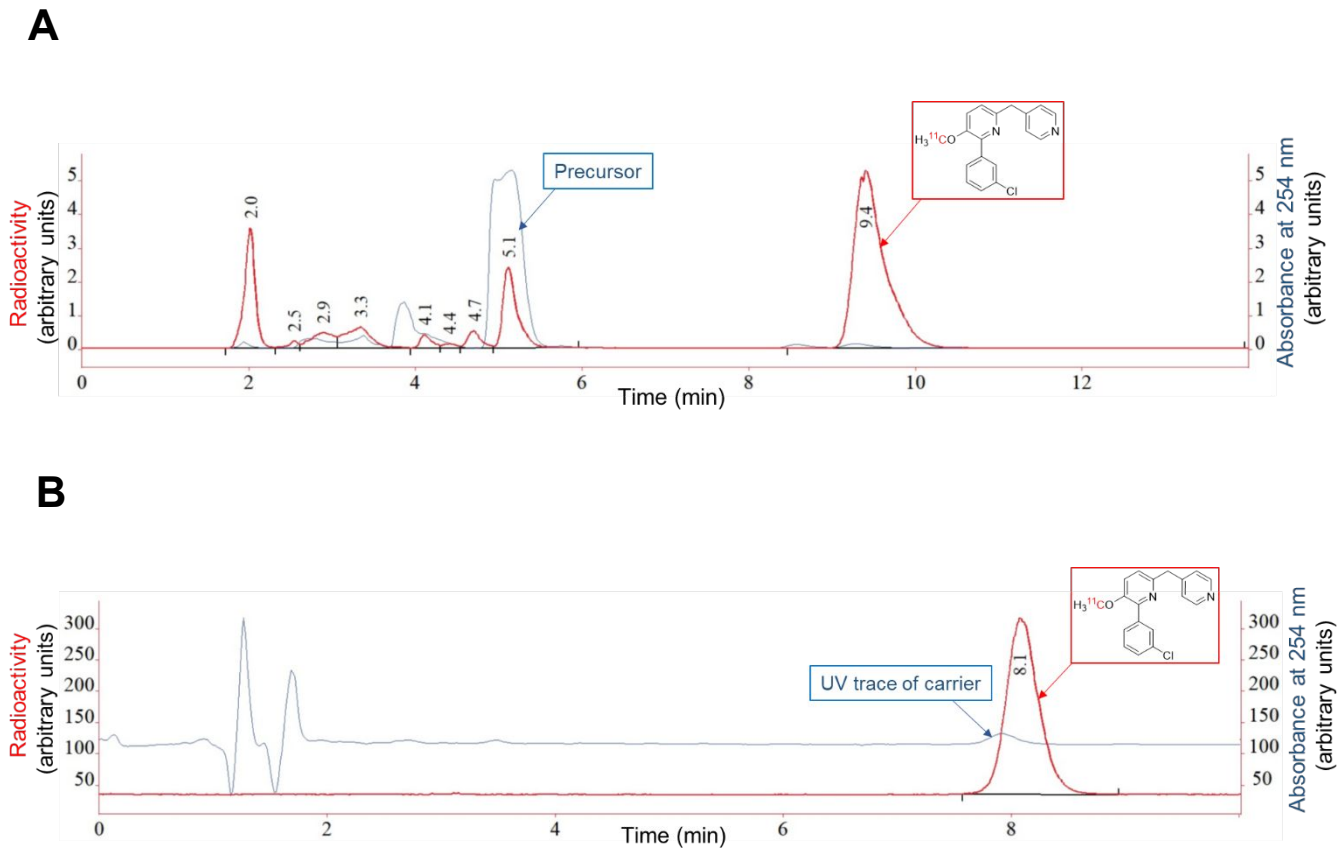


Figure S2. [^{11}C]**3** ([^{11}C]T1953) HPLC chromatograms. **A**: Preparative HPLC chromatogram. **B**: Analytical HPLC chromatogram.

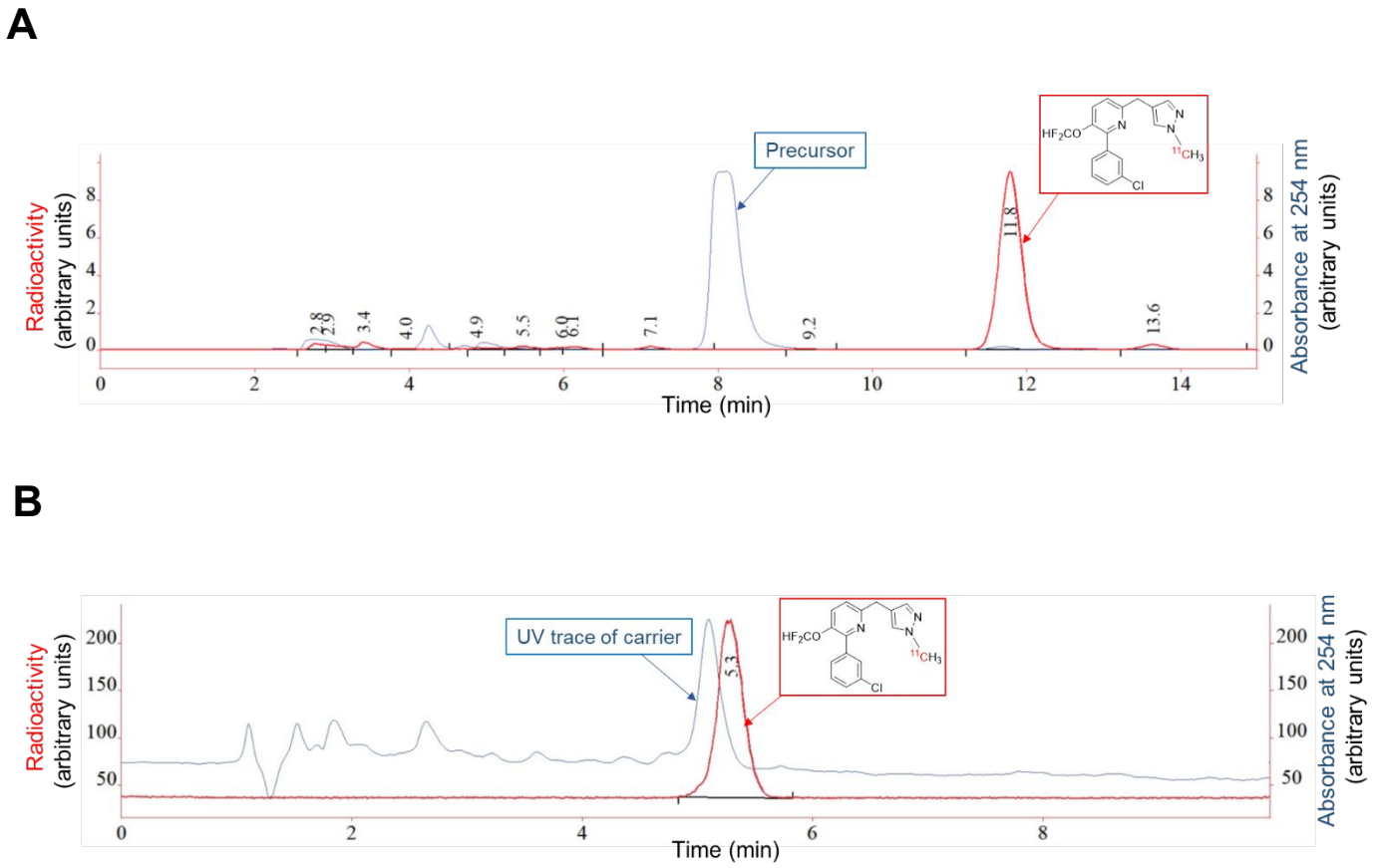


Figure S3. [^{11}C]**4** ([^{11}C]T2525) HPLC Chromatograms. **A**: Preparative HPLC chromatogram. **B**: Analytical HPLC chromatogram.

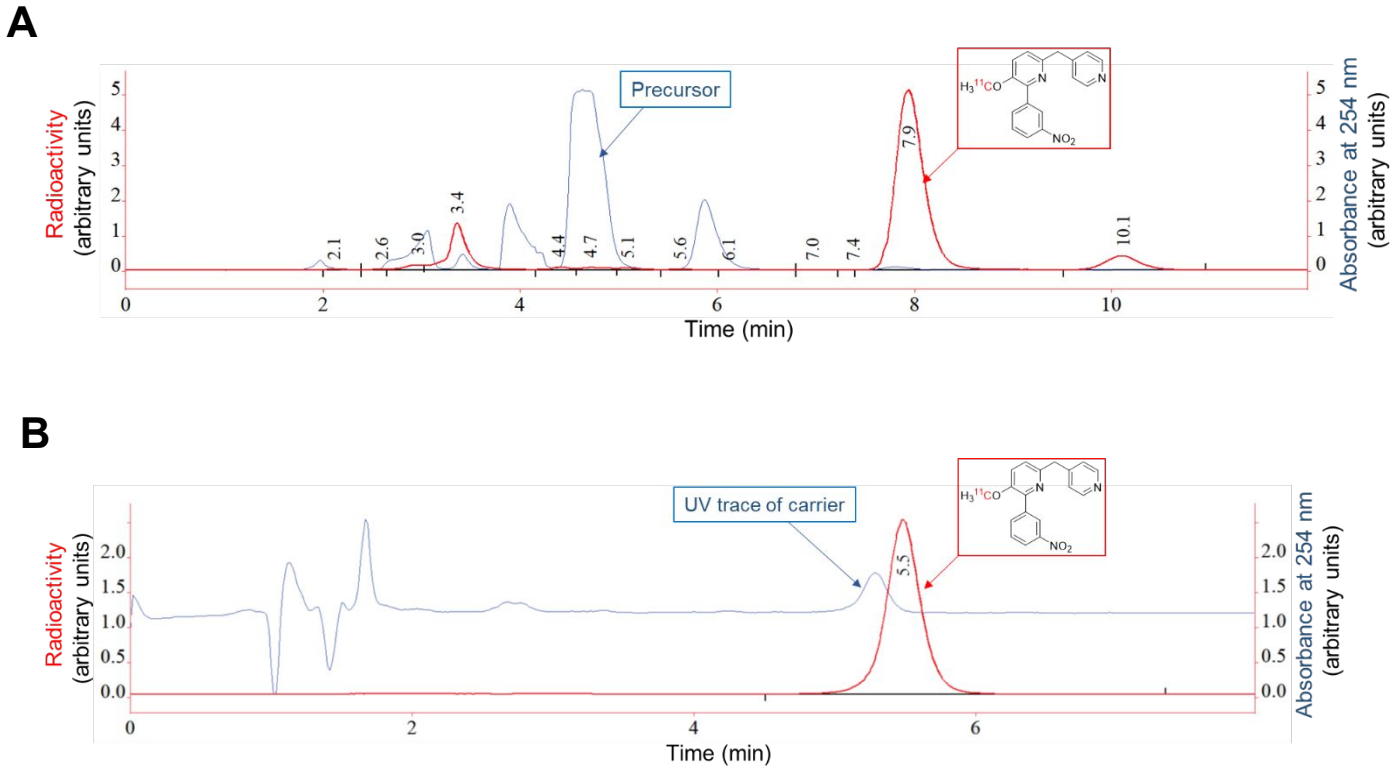


Figure S4. [^{11}C]**5** ([^{11}C]T1660) HPLC Chromatograms. **A**: Preparative HPLC chromatogram. **B**: Analytical HPLC chromatogram.

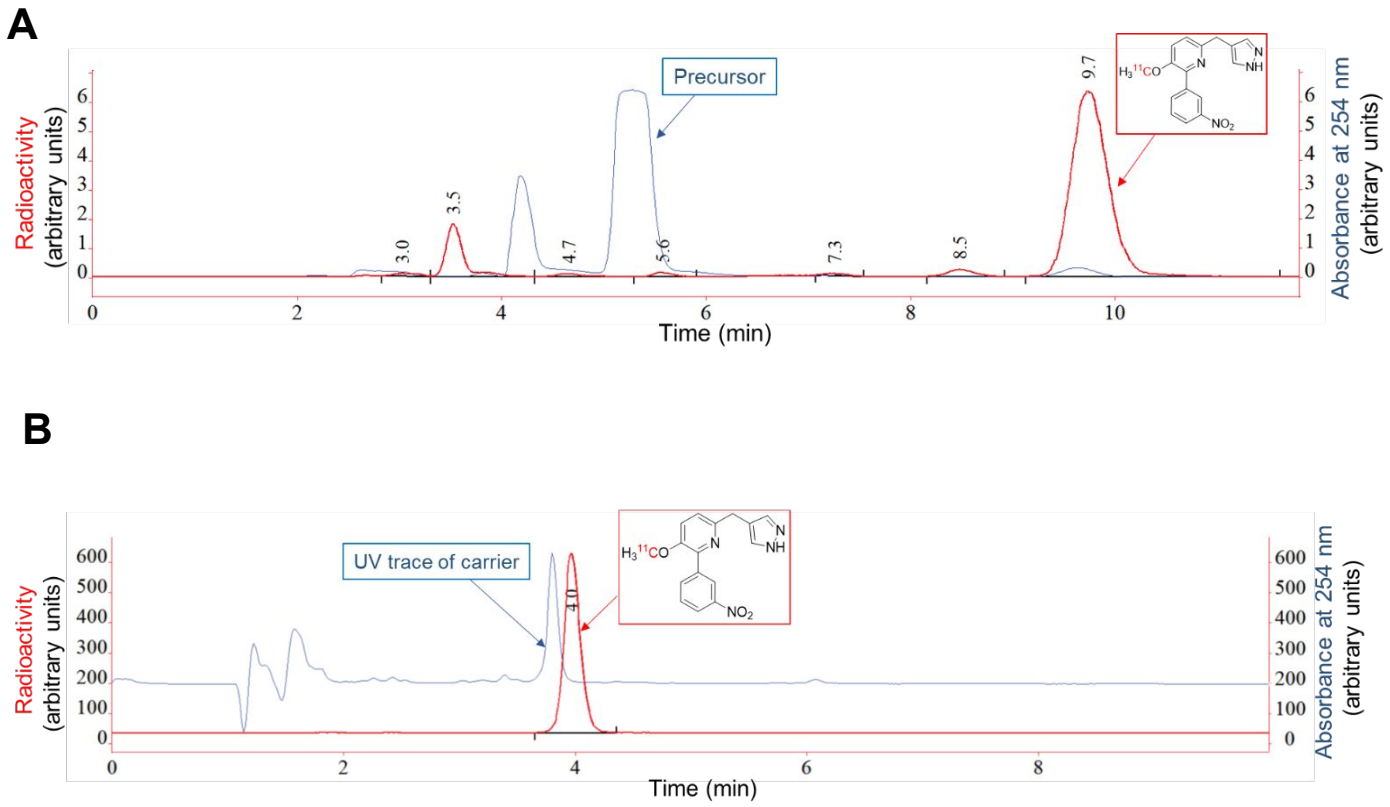


Figure S5. [^{11}C]**6 (**[^{11}C]T1650) HPLC Chromatograms: **A**: Preparative HPLC chromatogram. **B**: Analytical HPLC chromatogram.

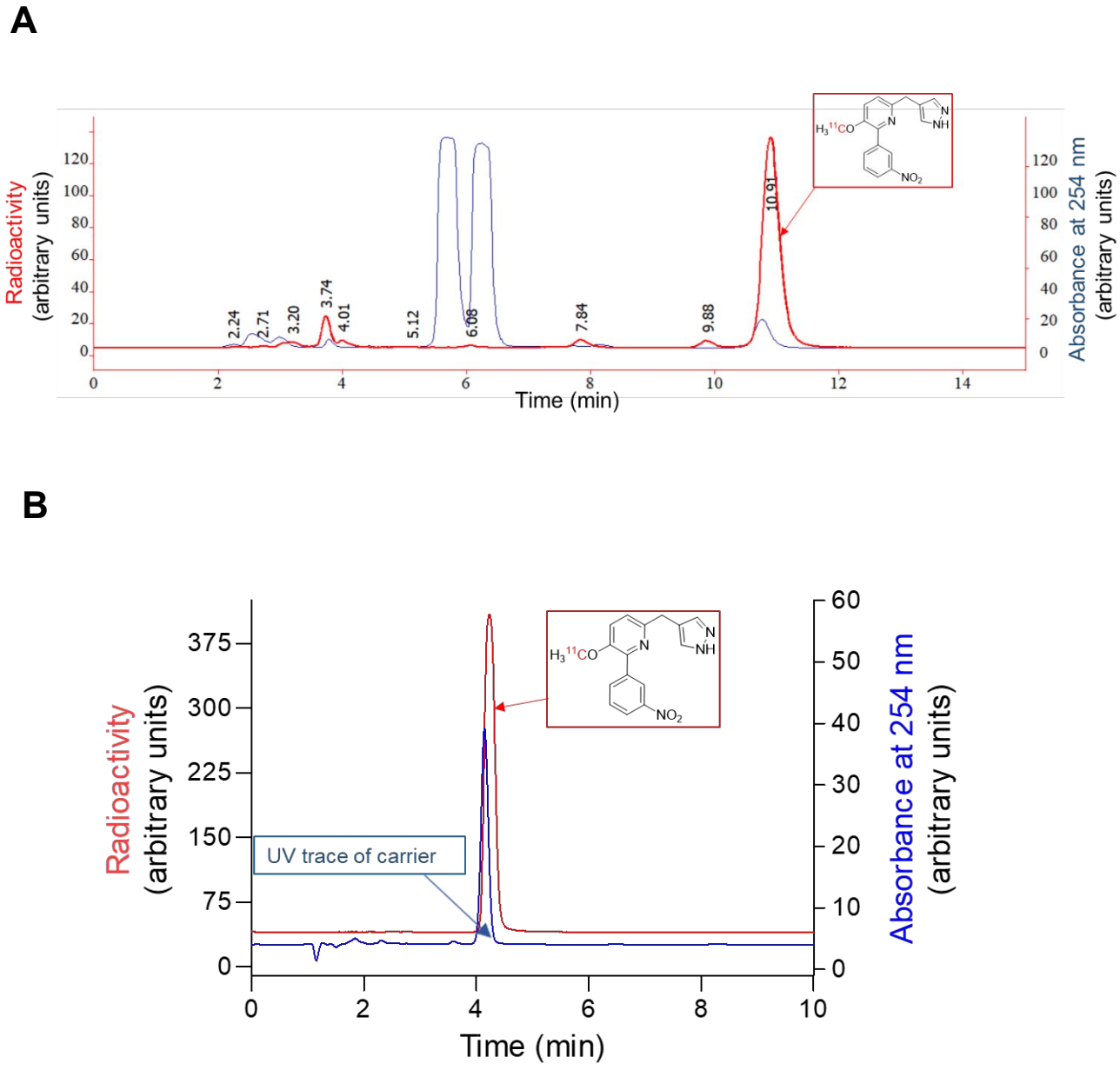


Figure S6. HPLC Chromatograms for [^{11}C]**6** ([^{11}C]T1650) production in a CGMP laboratory. **A**: Preparative HPLC chromatogram. **B**: Analytical HPLC chromatogram.

Table S3: Yields and A_m values for PDE4D radioligands evaluated in monkey.

Radioligand	Yield[a] (%)	A_m[b] (GBq/μmol at EOS)
[^{11}C]**3** ([^{11}C]T1953)	13 ± 2 (n = 4)	309 ± 90 (n = 4)
[^{11}C]**4** ([^{11}C]T2525)	17 ± 4 (n = 7)	228 ± 31 (n = 7)
[^{11}C]**5** ([^{11}C]T1660)	16 ± 5 (n = 5)	354 ± 93 (n = 3)
[^{11}C]**6** ([^{11}C]T1650)	20 ± 6 (n = 7)	229 ± 91 (n = 6)

[a] Estimated from starting cyclotron-produced [^{11}C]carbon dioxide. Some productions were used only for logD measurements; all others were used for PET imaging.
[b] Measured only for those productions used for PET imaging.

For human PET experiments [^{11}C]**6** ([^{11}C]T1650) was obtained in yields of 6.5 ± 2.5 GBq at end of synthesis (EOS) with molar activities of 265 ± 137 GBq/μmol (n = 4) from the amount of cyclotron-produced [^{11}C]carbon dioxide obtained from irradiation of the cyclotron target with a proton beam (16.5 MeV, 45 μA) for 40 min.

Table S4. Measured lipophilicities (m$\log D_{7.4}$) and monkey and human plasma free fractions (f_P) at baseline for tested radioligands.

Radioligand	m$\log D_{7.4}$[a] (n = 6)	Plasma free fraction (f_P)	
		Monkey[b]	Human control standard plasma
[^{11}C]**3** ([^{11}C]T1953)	3.45 ± 0.09	0.0115 & 0.0160	0.0077 & 0.0093
[^{11}C]**4** ([^{11}C]T2525)	3.07 ± 0.24	0.0144 & 0.0141	0.0109 & 0.0102
[^{11}C]**5** ([^{11}C]T1660)	3.38 ± 0.05	0.0498 & 0.0779	0.0271 & 0.0406
[^{11}C]**6** ([^{11}C]T1650)	2.89 ± 0.03	0.0666 ± 0.0067[c]	0.0341 ± 0.0044[b]

[a] Measured with a method based on partition of formulated radioligand between n-octanol and sodium phosphate buffer (0.15 M; pH 7.4), as described previously (1).
[b] Values for particular monkey PET experiments are given in the main text.
[c] The average SD from 6 separate studies

Notes:

1. Plasma free fraction was simultaneously determined in the experimental monkey plasma and in human control standards.
    a. Monkey plasma: Plasma was prepared by centrifugation of blood taken from the experimental monkey before its injection with the radioligand.
    b. Human control standard: pooled human plasma that had been aliquoted into Eppendorf tubes and individually stored at – 70 °C.
2. Plasma free fraction was measured by the addition of radiochemically pure radioligand (1.11 – 2.22 kBq) to 650 μL of non-radioactive plasma. After mixing and incubation for 10 min at room temperature, the free fraction was determined by ultrafiltration (Centrifree; Millipore, Billerica, Massachusetts) as previously described (2).

3. In order to eliminate inter-monkey variability, we used the free fraction values as determined by using the frozen human plasma, to better highlight the differences between the free fractions of the various radioligands.

References

1. Zoghbi SS, Anderson KB, Jenko KJ, Luckenbaugh DA, Innis RB and Pike VW. On quantitative relationships between drug-like compound lipophilicity and plasma free fraction in monkey and human. *J Pharm Sci* 2012; *101*, 1028−1039.

2. Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. Evaluation of ultrafiltration for the free-fraction determination of single photon emission computed tomography (SPECT) radiotracers: ß-CIT, IBF, and iomazenil. *J Pharm Sci* 1994; *83*: 1014–1019.

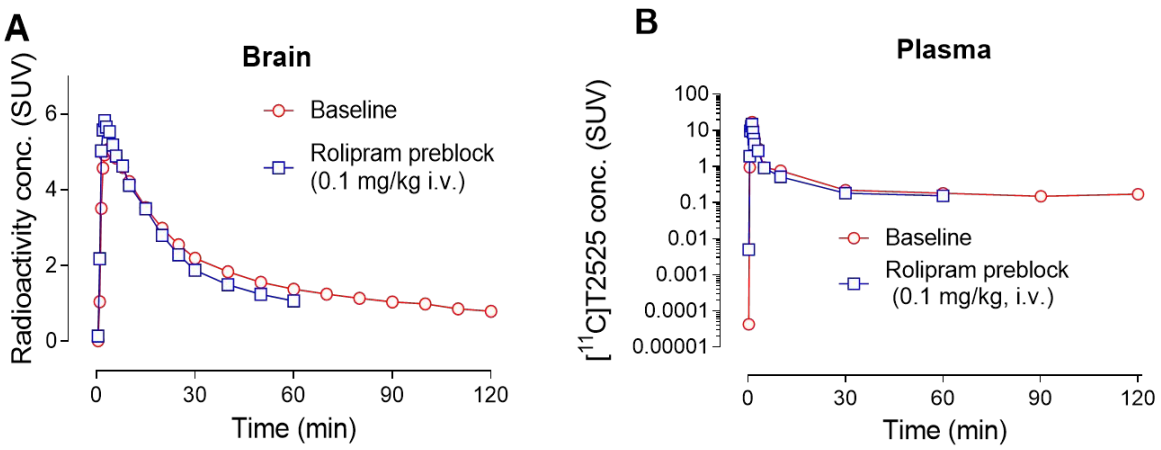


Figure S7. Data from PET experiments with [^{11}C]**4** ([^{11}C]T2525) in monkey. Time-activity curves for whole brain at baseline and in a subsequent experiment in which rolipram (0.1 mg.kg) was administered intravenously at 5 min before [^{11}C]**4** to preblock PDE4D enzyme (**A**). Time course of unchanged [^{11}C]**4** in plasma during the baseline and rolipram preblock experiment (**B**). For these experiments, a single male monkey (13.9 kg) was injected intravenously with [^{11}C]**4** (321 MBq, A_m = 216 GBq/µmol) at baseline and with [^{11}C]**4** (283 MBq, A_m = 275 GBq/µmol) for the rolipram pre-block experiment.

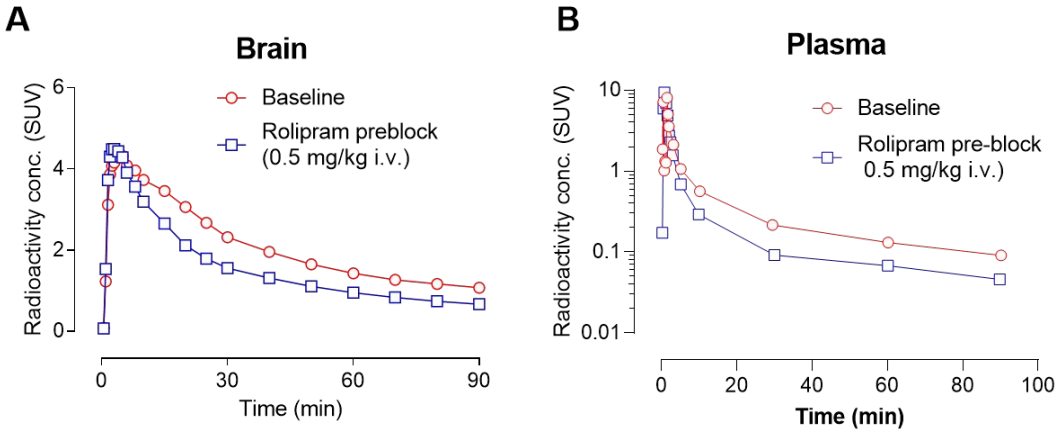


Figure S8. Data from PET experiments with [^{11}C]**3** ([^{11}C]T1953) in monkey. Time-activity curves for whole brain at baseline and in a subsequent experiment in which rolipram (0.5 mg.kg) was administered intravenously at 5 minutes before [^{11}C]**3** to preblock PDE4D enzyme (**A**). Time course of unchanged [^{11}C]T1953 in plasma during the baseline and rolipram preblock experiment (**B**). For these experiments, a single male monkey (13.8 kg) was injected intravenously with [^{11}C]**3** (248 MBq; A_m = 185 GBq/μmol) at baseline and with [^{11}C]**3** (289 MBq, A_m = 162 GBq/μmol) for the rolipram pre-block experiment.

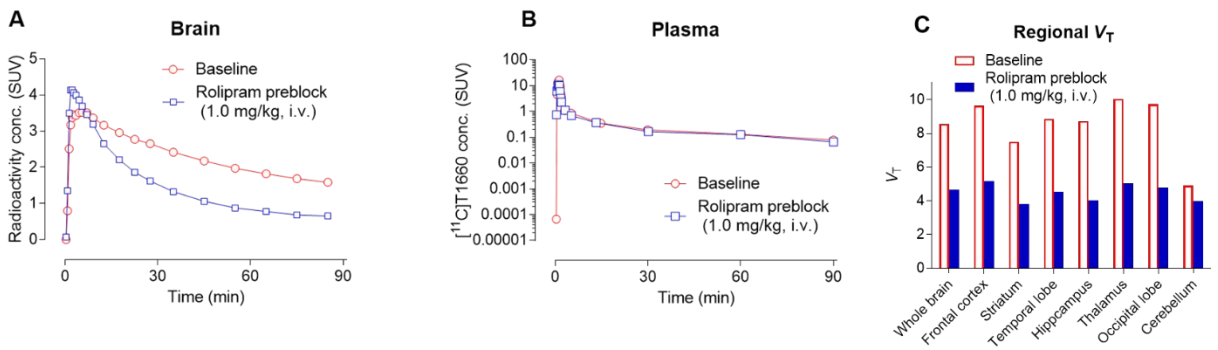


Figure S9. Data from PET experiments with [^{11}C]**5** ([^{11}C]T1660) in monkey. Time-activity curves for whole brain at baseline and in a subsequent experiment in which rolipram (1.0 mg/kg) was administered intravenously at 5 minutes before [^{11}C]**5** to preblock PDE4D enzyme (**A**). Time course of unchanged [^{11}C]**5** in plasma during the baseline and rolipram preblock experiment (**B**). Brain regional V_T values at baseline and after rolipram preblock (**C**). For these experiments, a single male monkey (10.9 kg) was injected intravenously with [^{11}C]**5** (321 MBq, A_m = 216 GBq/μmol) at baseline and with [^{11}C]**5** (283 MBq, A_m = 275 GBq/μmol) for the rolipram pre-block experiment.

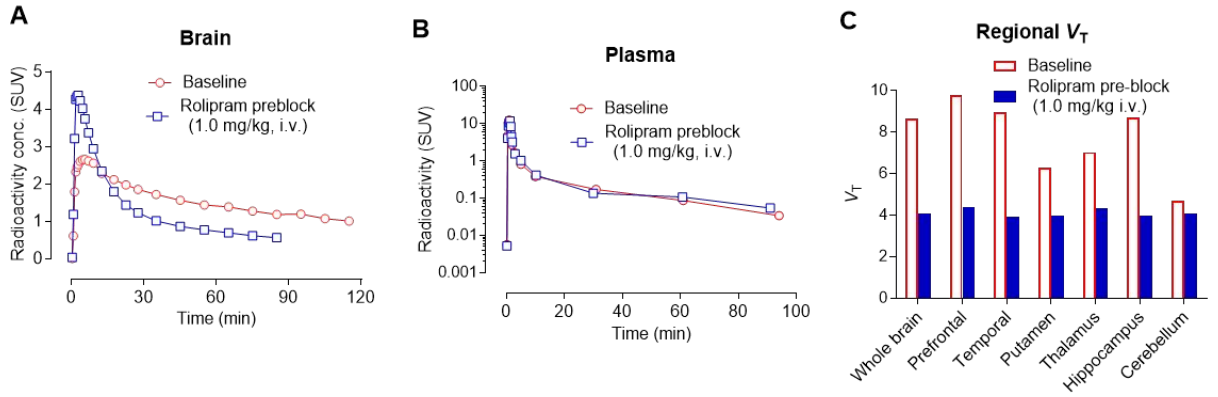


Figure S10. Data from PET experiments with [^{11}C]**6** ([^{11}C]T1650) in monkey. Time-activity curves for whole brain at baseline and in a subsequent experiment in which rolipram (1.0 mg/kg) was administered intravenously at 5 minutes before [^{11}C]**6** to preblock PDE4D enzyme (**A**). Time course of unchanged [^{11}C]**6** in plasma during the baseline and rolipram preblock experiment (**B**). Brain regional V_T values at baseline and after rolipram preblock (**C**). For these experiments, a single male monkey (12.9 kg) was injected intravenously with [^{11}C]**6** (316 MBq, A_m = 65 GBq/μmol) at baseline and with [^{11}C]**6** (349 MBq, A_m = 108 GBq/μmol) for the rolipram pre-block experiment.

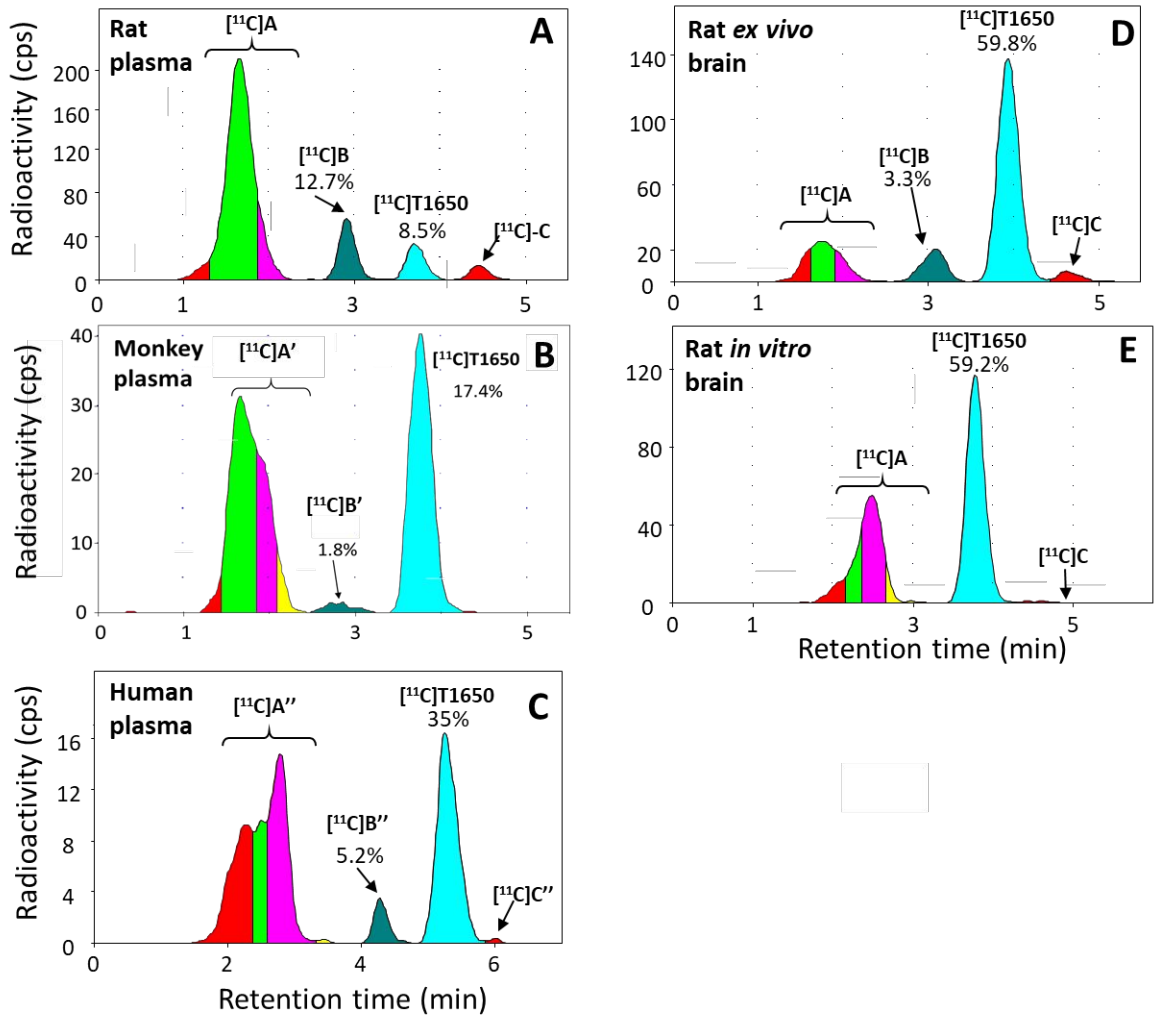


Figure S11. HPLC analyses of the radiometabolites of [^{11}C]**6** ([^{11}C]T1650) generated over 30 min in various tissues: plasma sampled during PET experiments in rat (**A**), monkey (**B**), and human (**C**), rat brain *ex vivo* (**D**), and fresh rat brain homogenate *in vitro* (**E**). Rat brain *in vitro* gave a similar radiometabolite profile to rat brain *ex vivo* but did not produce radiometabolite [^{11}C]B. Rat brain radiometabolites therefore had both peripheral and central origin. [^{11}C]**B"** was observed in human plasma. Radiometabolite profiles for rat, monkey, and human plasma, sampled during PET experiments, were quite similar.

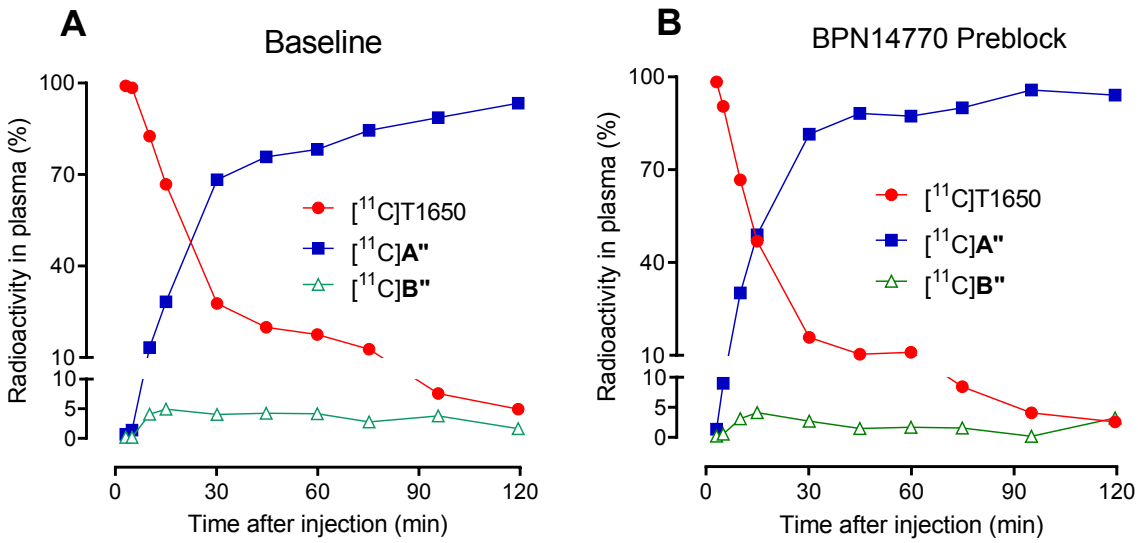


Figure S12. Time-courses for radioactive species in plasma of a single human subject following intravenous administration of [^{11}C]**6** ([^{11}C]T1650) at baseline (**A**) and in a BPN14770 preblock experiment (**B**). Under each condition, [^{11}C]**6** declines quite rapidly, the group of polar radiometabolites [^{11}C]**A"**, rise, and the lipophilic radiometabolite [^{11}C]**B"**, which may penetrate the blood-brain barrier, stays relatively low and constant.

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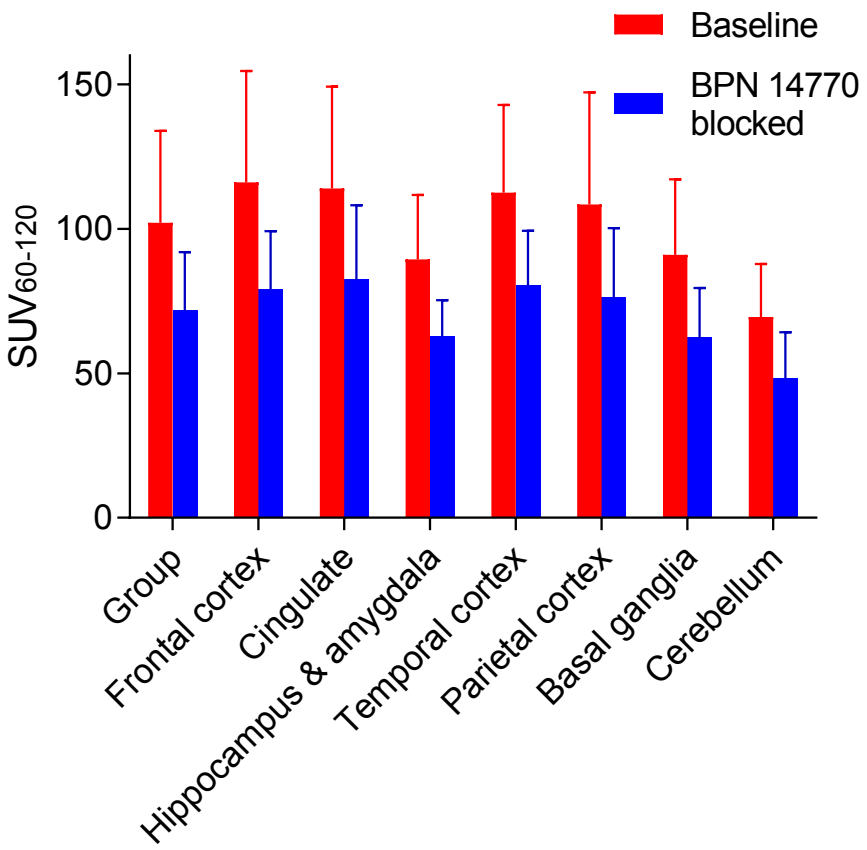
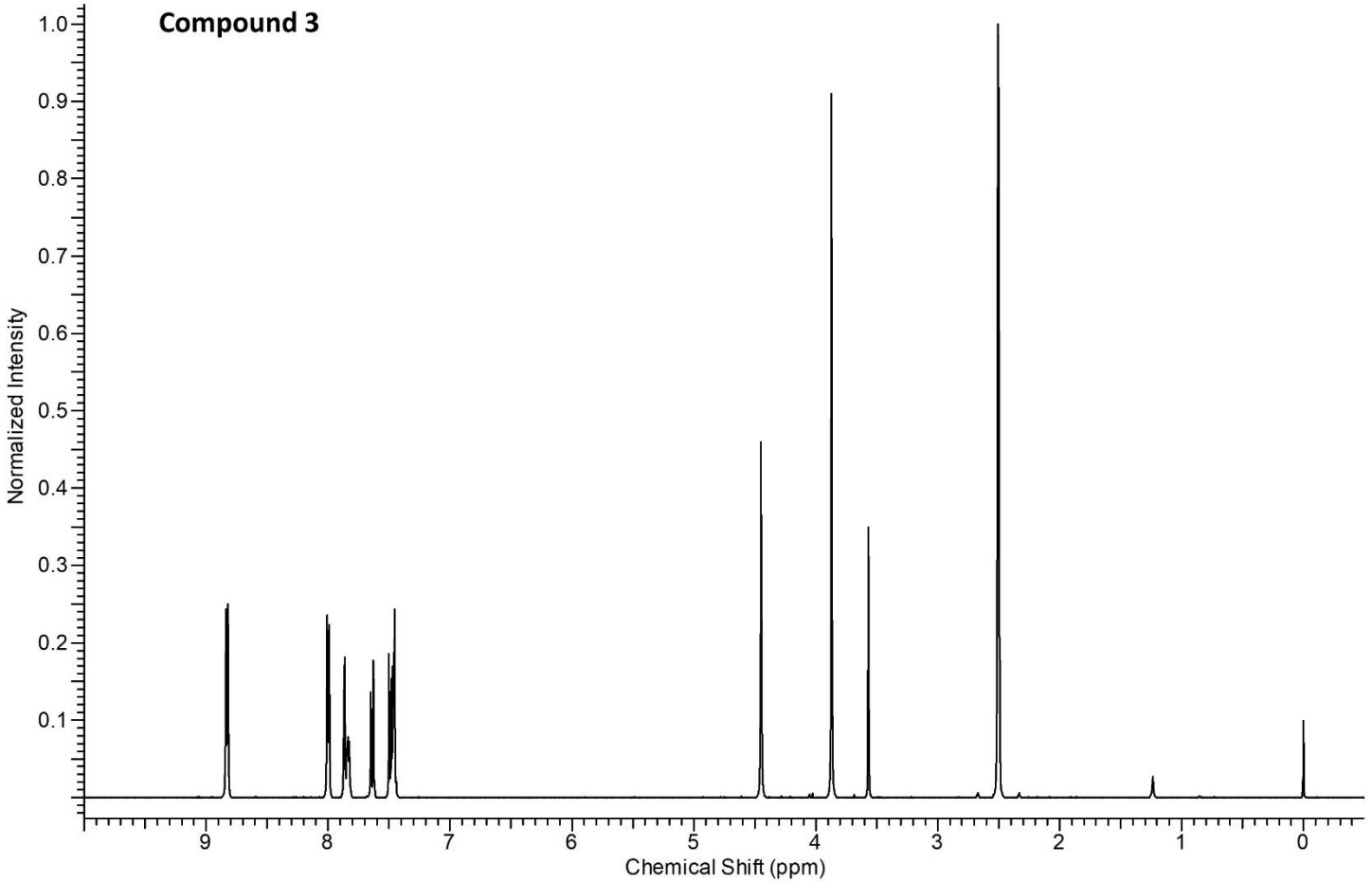
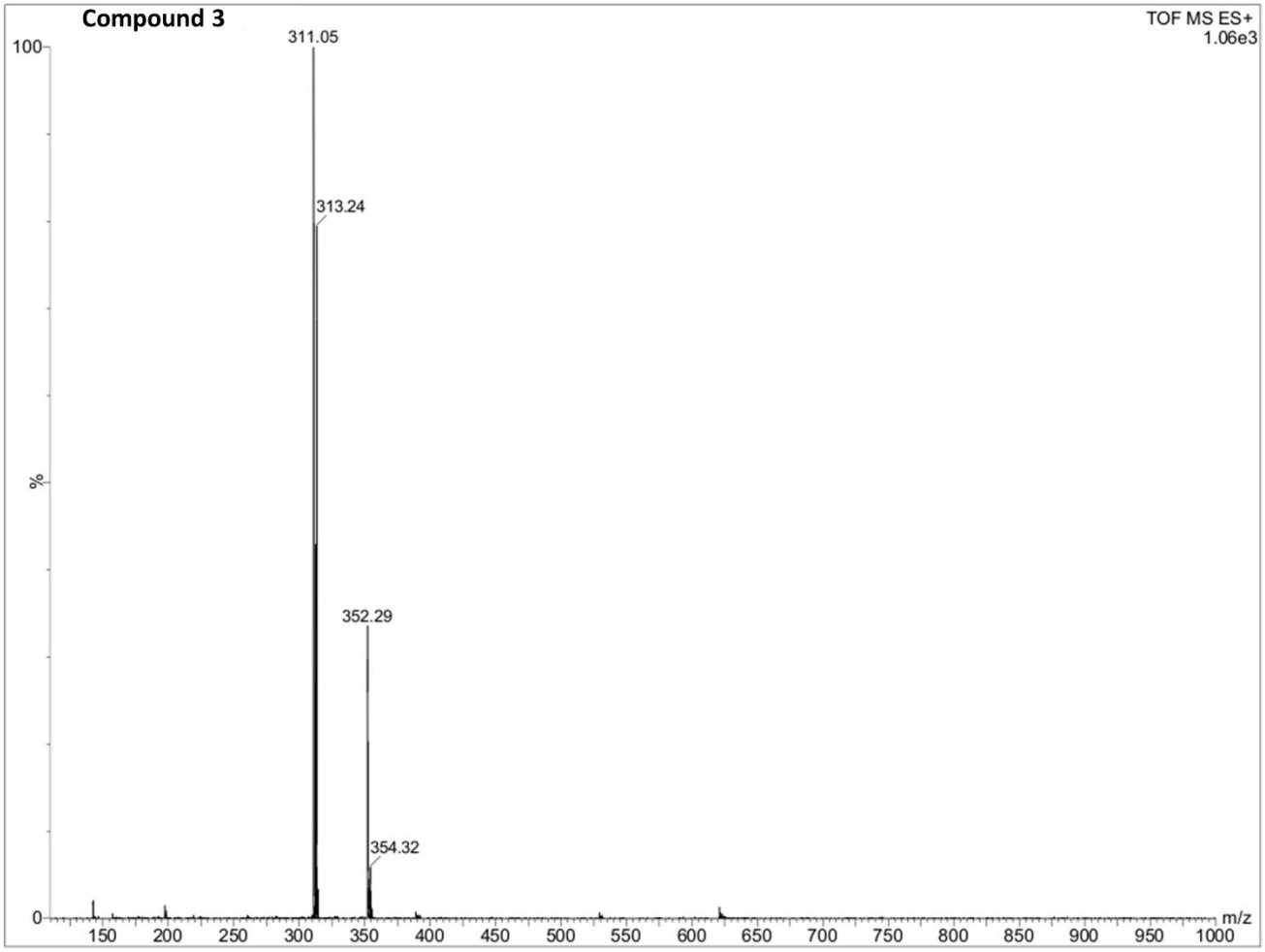


Figure S13. Average area (± range) of *AUC* for SUV from 60 to 120 minutes for two human participants. A significant difference ($p < 0.05$) was observed between the baseline and BPN14770 blocked scan.

Appendix 1. Characterization data for compounds 3–10.

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m/z

Sample Name: T-1953

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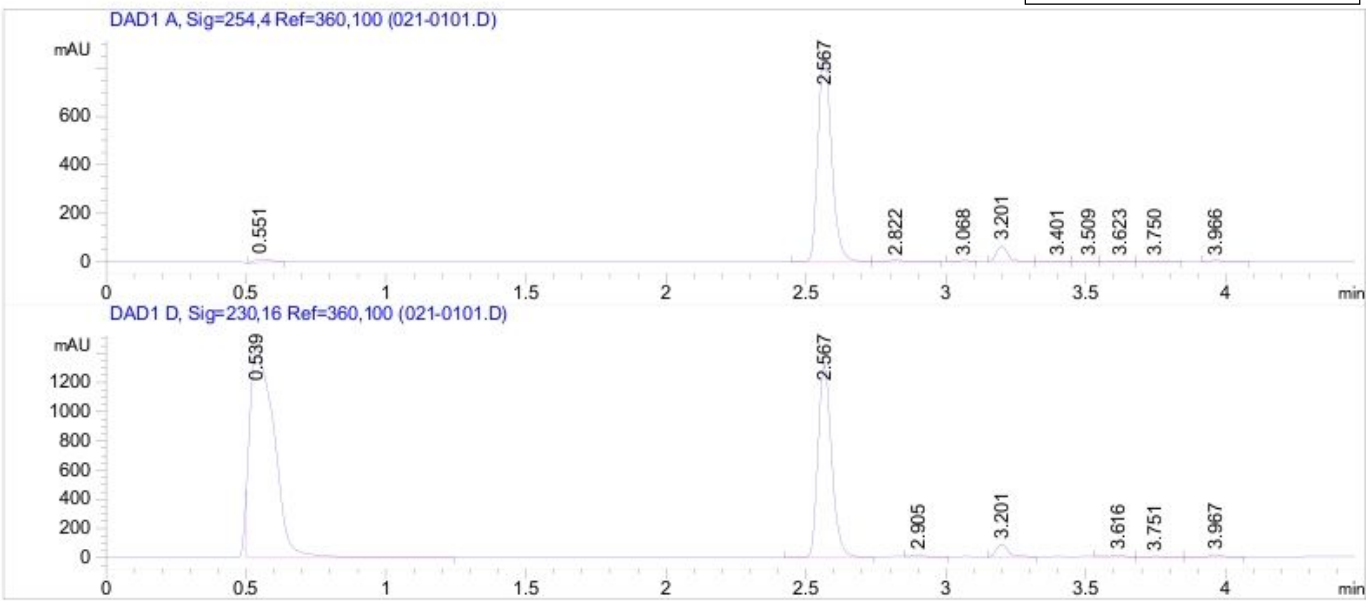
Compound 3

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Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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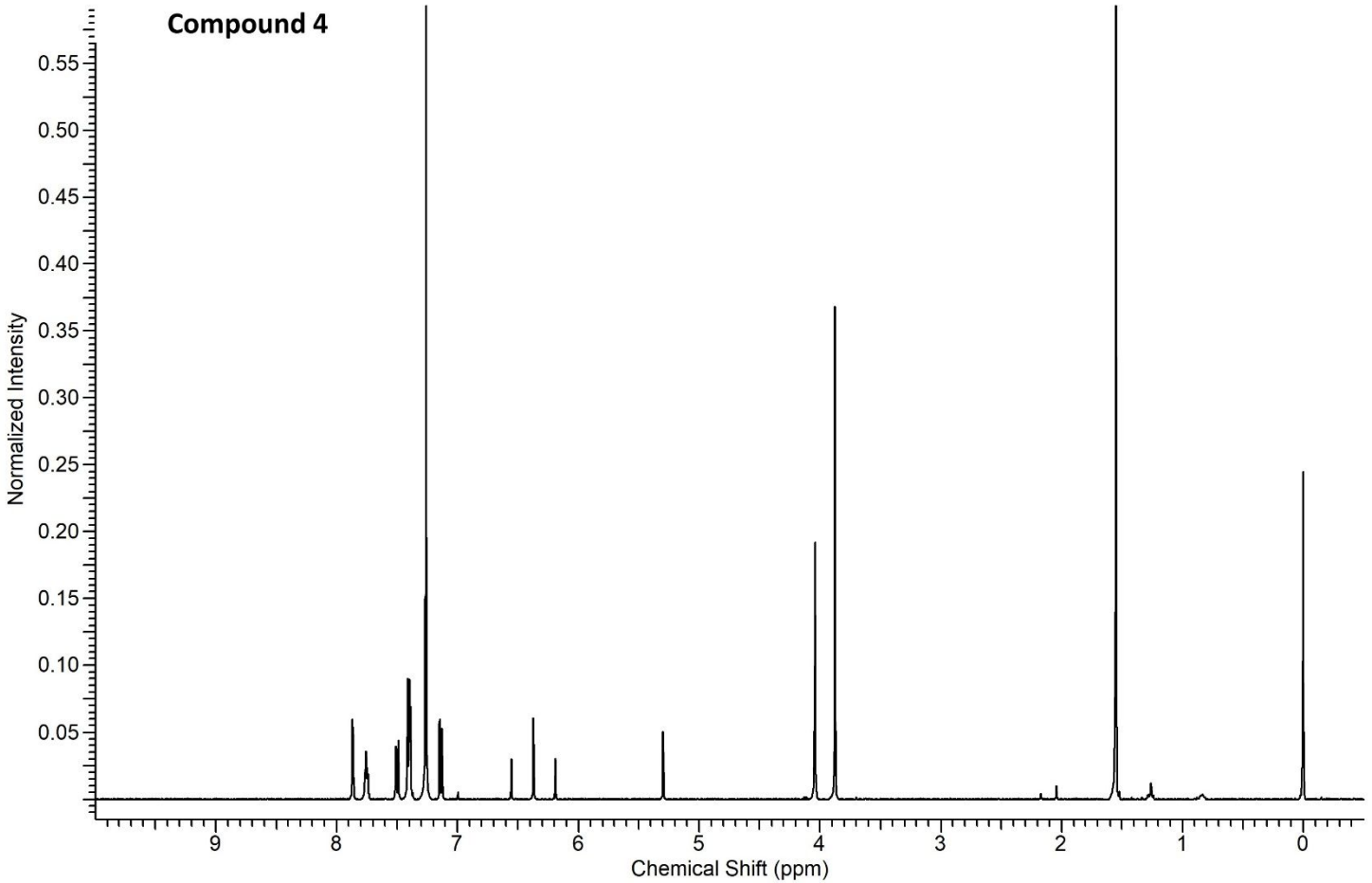
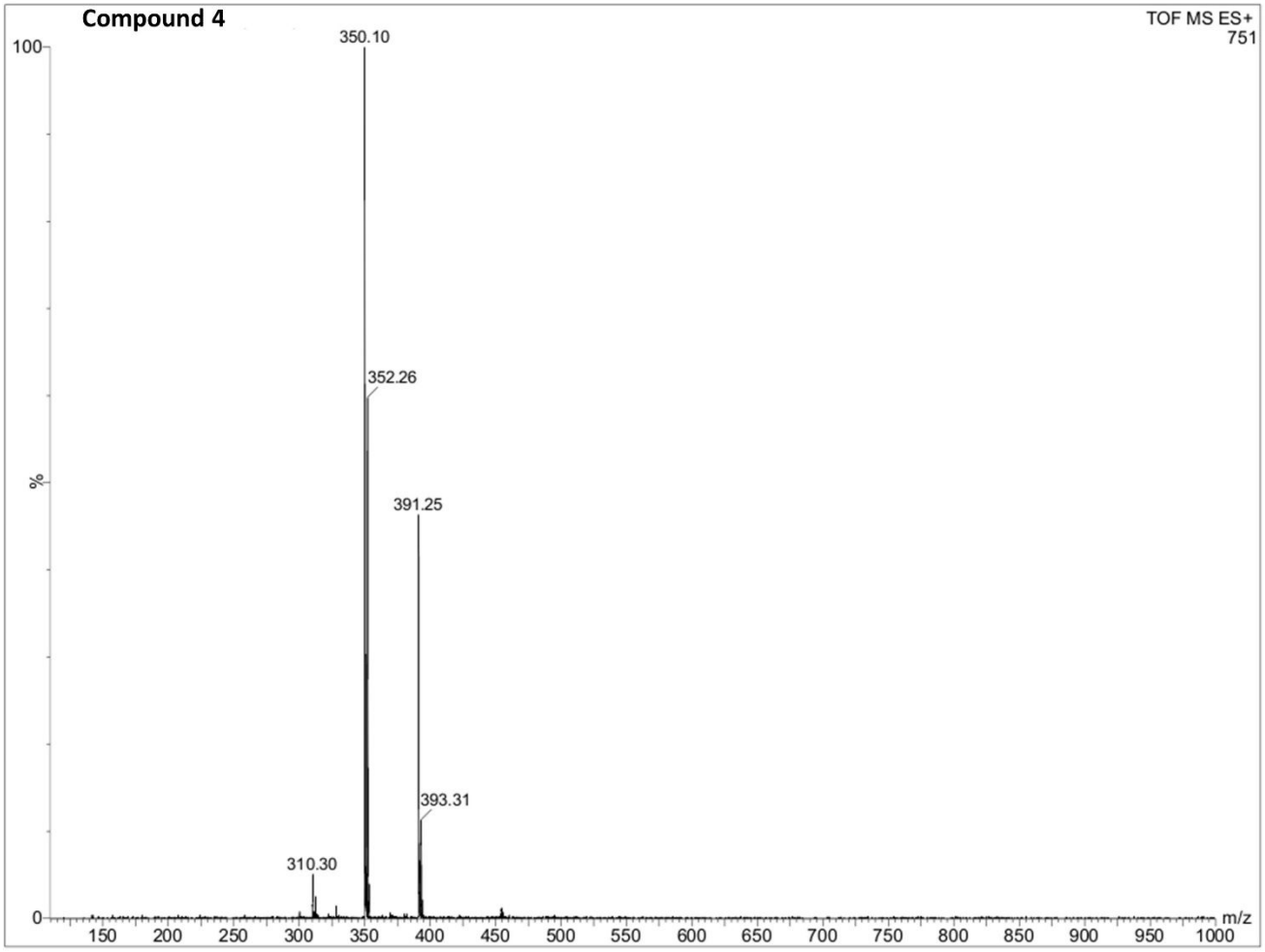
4	3.068	BB	0.0418	6.63510	2.59704	0.2072
5	3.201	BB	0.0442	170.79424	61.86639	5.3340
6	3.401	BB	0.0454	5.10278	1.78195	0.1594
7	3.509	BB	0.0378	5.30663	2.22504	0.1657
8	3.623	BB	0.0508	8.71265	2.61982	0.2721
9	3.750	BB	0.0617	5.94552	1.45314	0.1857
10	3.966	BB	0.0493	11.28317	3.52795	0.3524

Sample Name: T-1953

Compound 3

```
    ==================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   1
    Acq. Instrument : Instrument 1                    Location : Vial 21
    Injection Date  : 1/9/2020 8:32:43 AM                  Inj :   1
                                                     Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M (
                      Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ==================================================================

    Totals :                  3201.96345  964.94425


    Signal 2: DAD1 D, Sig=230,16 Ref=360,100

    Peak RetTime Type  Width     Area       Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.539 BB    0.1015 8646.67383 1452.00574  64.4963
       2   2.567 BB    0.0515 4459.79590 1318.84167  33.2660
       3   2.905 BB    0.0395   12.81956    5.43780   0.0956
       4   3.201 BB    0.0457  242.38814   84.03511   1.8080
       5   3.616 BB    0.0512   16.99269    5.05645   0.1268
       6   3.751 BB    0.0648    5.77079    1.27546   0.0430
       7   3.967 BB    0.0491   22.01890    6.91523   0.1642

    Totals :                  1.34065e4  2873.56746


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

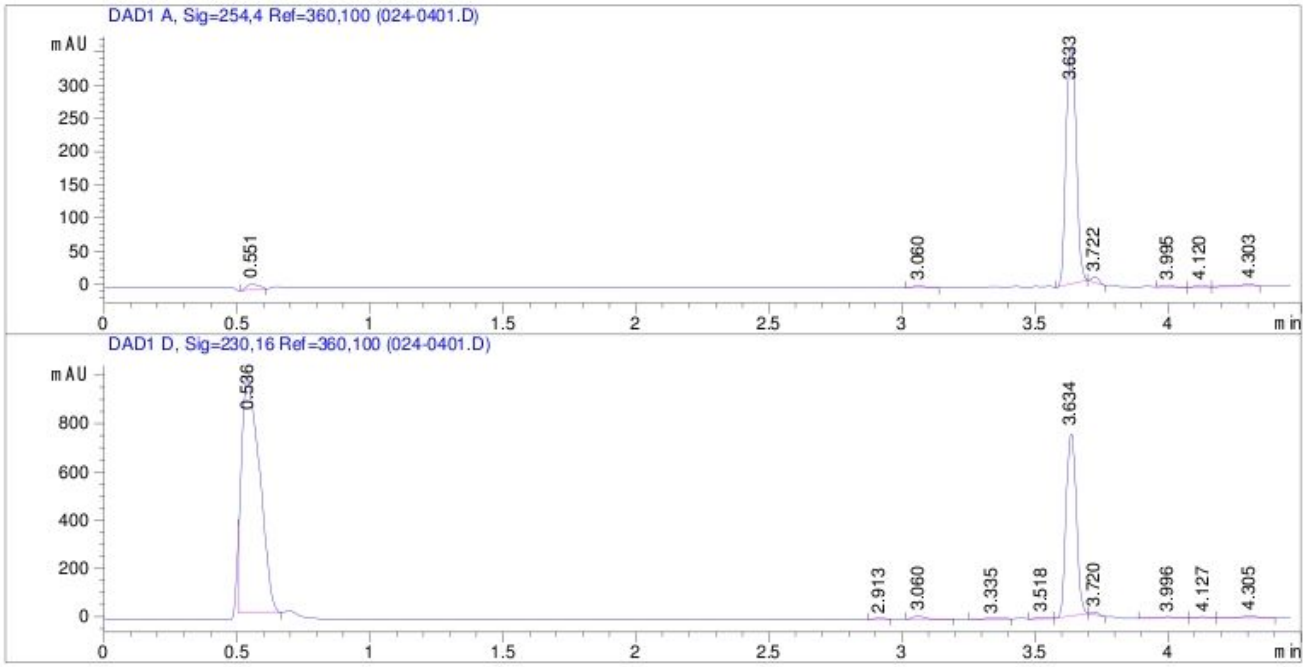

Compound 4
Normalized Intensity
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Compound 4
TOF MS ES+
751
100
%
0
350.10
352.26
391.25
393.31
310.30
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Sample Name: T-2525

```
    ====================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   4
    Acq. Instrument : Instrument 1                    Location : Vial 24
    Injection Date  : 1/9/2020 8:50:34 AM                 Inj :   1
                                                    Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M
                      (Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ====================================================================
```

Compound 4




```
    ====================================================================
                           Area Percent Report
    ====================================================================

    Sorted By             :      Signal
    Multiplier:                   :      1.0000
    Dilution:                     :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.551 BB    0.0447   28.58870    9.07978   2.7681
       2   3.060 BB    0.0488   11.43199    3.62749   1.1069
       3   3.633 BB    0.0427  943.83051  359.08966  91.3854
       4   3.722 BB    0.0327   14.17191    7.29682   1.3722
       5   3.995 BB    0.0418    7.87900    3.08767   0.7629
       6   4.120 BB    0.0422    7.94757    3.07515   0.7695
       7   4.303 BB    0.0724   18.95312    3.66206   1.8351
```

Sample Name: T-2525

```
    ================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   4
    Acq. Instrument : Instrument 1                    Location : Vial 24
    Injection Date  : 1/9/2020 8:50:34 AM                 Inj :   1
                                                    Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M
                      (Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ================================================================
```

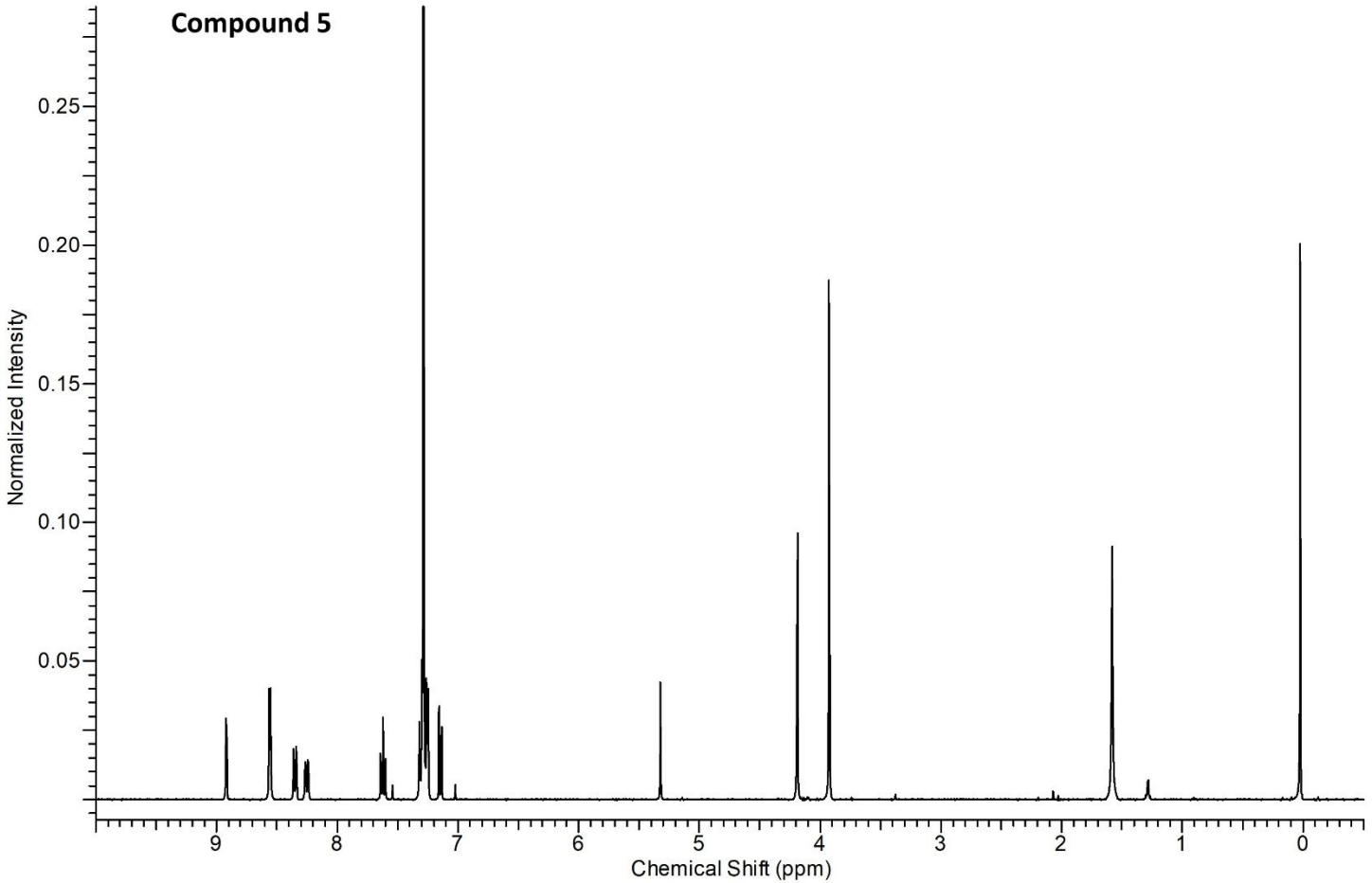
Compound 4

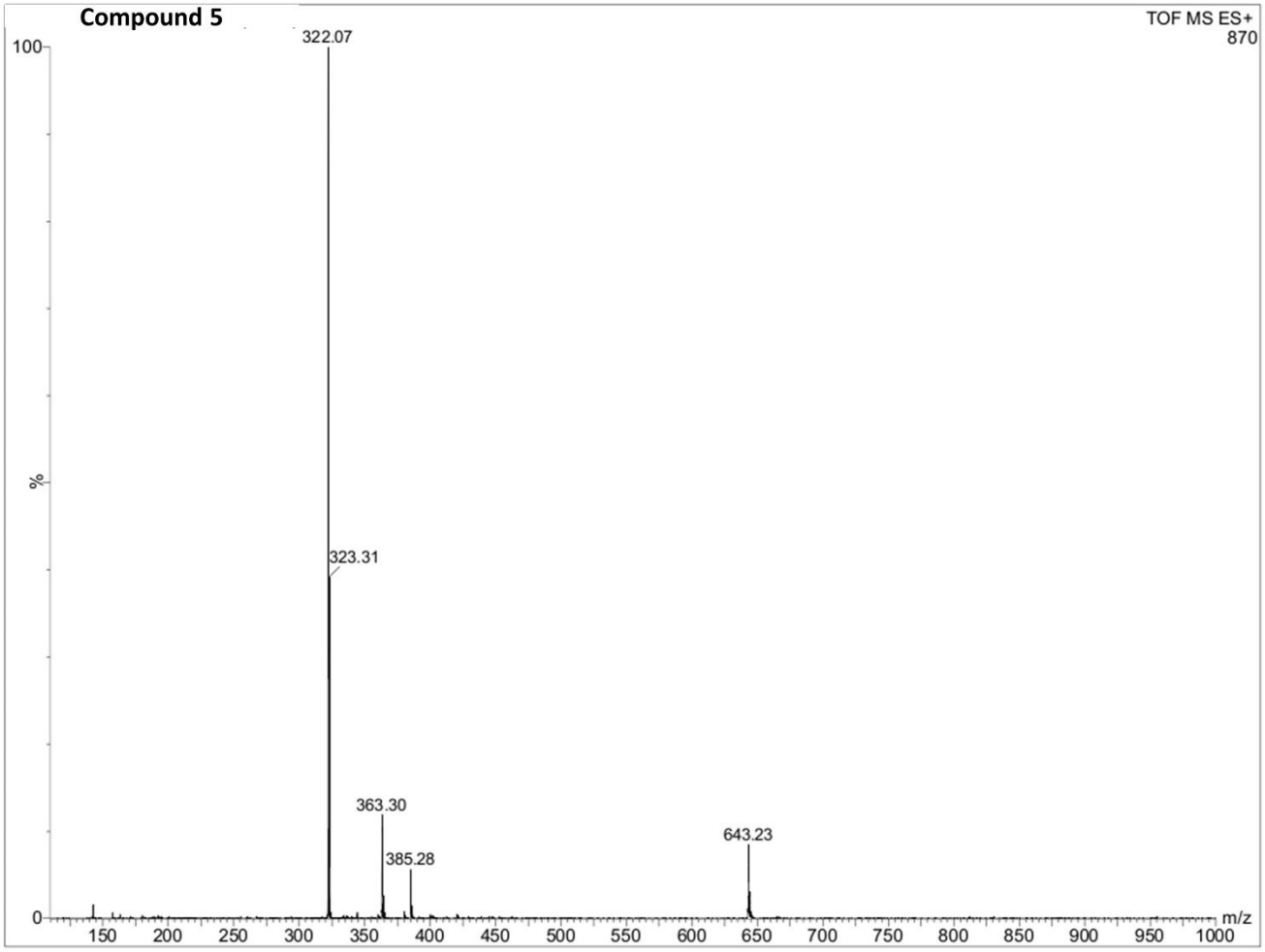
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1032.80279	388.91863	

Signal 2: DAD1 D, Sig=230,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.536	BB	0.0806	4568.00830	976.36768	67.8306
2	2.913	BB	0.0404	10.30285	4.22576	0.1530
3	3.060	BB	0.0469	37.43121	12.50152	0.5558
4	3.335	BB	0.0528	9.98064	2.72056	0.1482
5	3.518	BB	0.0431	7.71517	2.89877	0.1146
6	3.634	BB	0.0428	2017.14099	763.22974	29.9526
7	3.720	BB	0.0315	15.51579	8.40579	0.2304
8	3.996	BB	0.0582	19.94030	5.03152	0.2961
9	4.127	BB	0.0438	11.70332	4.29360	0.1738
10	4.305	BB	0.0592	36.70059	9.07931	0.5450
Totals :				6734.43916	1788.75425	

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

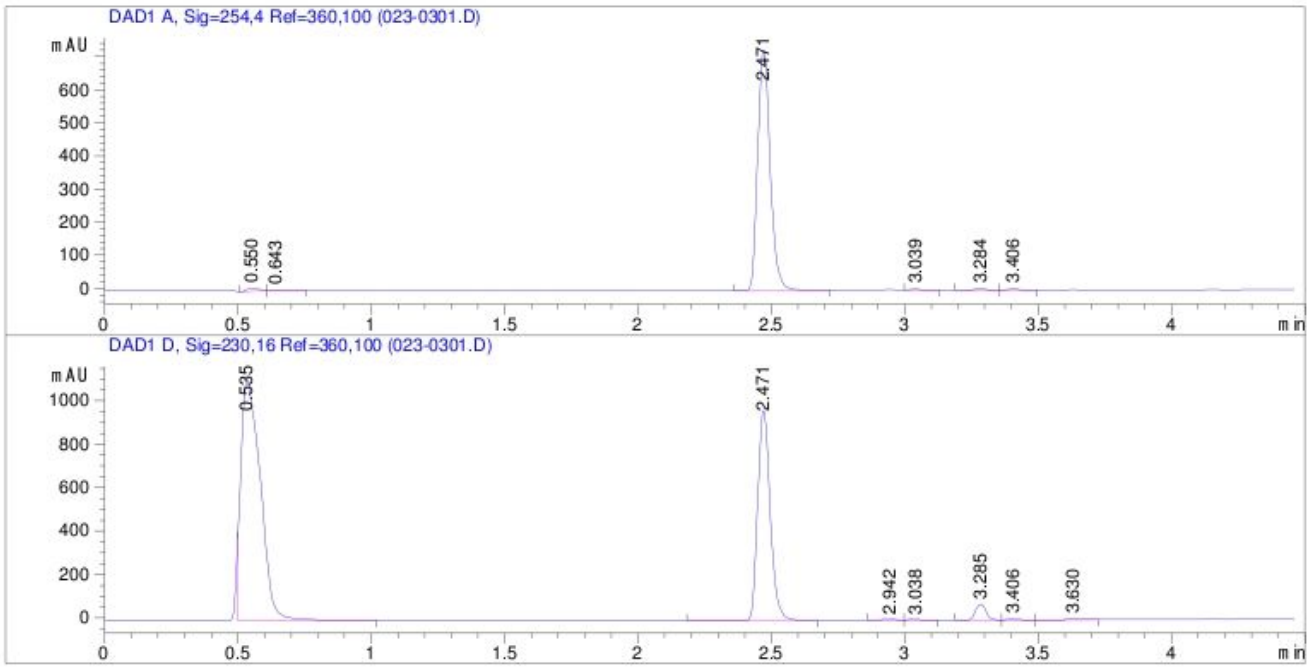

Compound 5
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Compound 5
TOF MS ES+
870
100
%
0
322.07
323.31
363.30
385.28
643.23
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Sample Name: T-1660

```
    ======================================================================
    Acq. Operator    : Tetra Discovery Partners       Seq. Line :   3
    Acq. Instrument  : Instrument 1                    Location : Vial 23
    Injection Date   : 1/9/2020 8:44:35 AM                 Inj :   1
                                                     Inj Volume : 5.0 µl
    Sequence File    : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
    Method           : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M
                       (Sequence Method)
    Last changed     : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info      : Standard method for small molecule separation

    ======================================================================
```

Compound 5

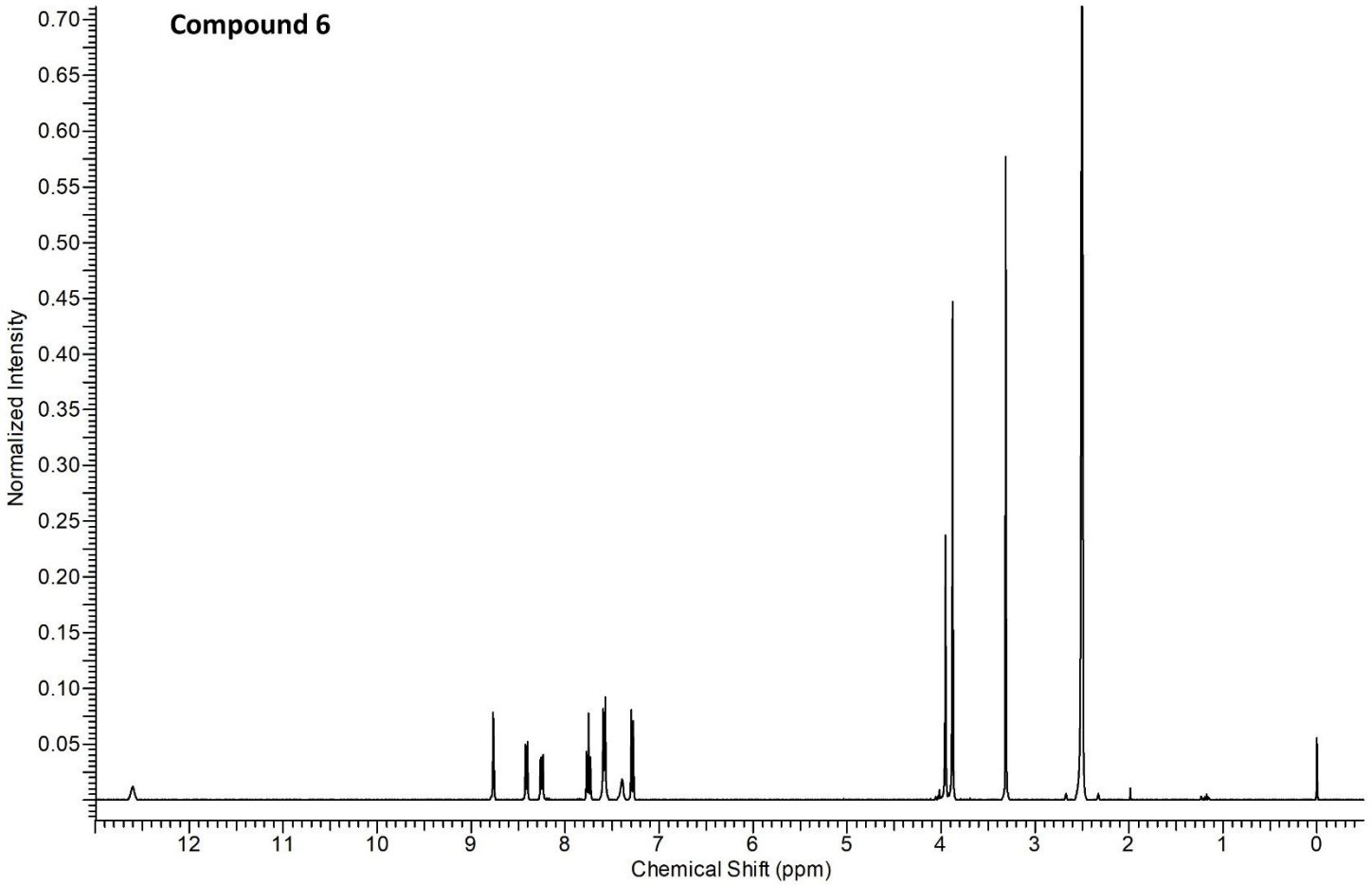


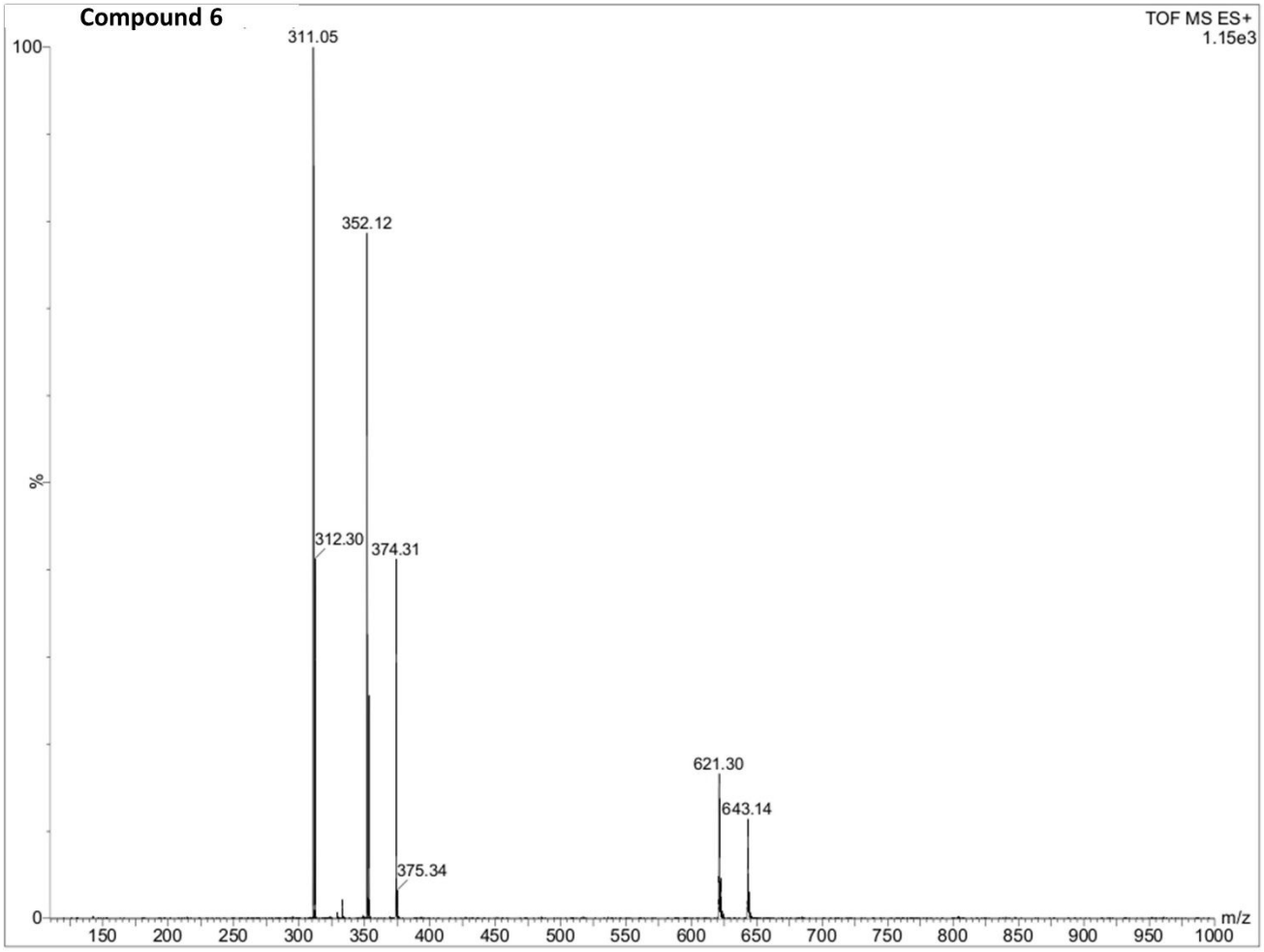

```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier:                   :      1.0000
    Dilution:                     :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]       [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.550 BB    0.0451   29.94461    9.40484   1.2259
       2   0.643 BB    0.0432    7.40898    2.45612   0.3033
       3   2.471 BB    0.0497 2367.99854  733.60571  96.9457
       4   3.039 BB    0.0443    7.79230    2.81743   0.3190
       5   3.284 BB    0.0501   15.08457    4.62256   0.6176
       6   3.406 BB    0.0458   14.37478    4.95661   0.5885
```

Sample Name: T-1660

```
    ================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   3
    Acq. Instrument : Instrument 1                    Location : Vial 23
    Injection Date  : 1/9/2020 8:44:35 AM                 Inj :   1
                                                     Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M
                      (Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ================================================================

    Totals :                  2442.60379  757.86327


    Signal 2: DAD1 D, Sig=230,16 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.535 BB    0.0724 5576.53760 1114.38977  62.1930
       2   2.471 BB    0.0497 3153.35059  975.58374  35.1681
       3   2.942 BB    0.0628    9.83256    2.45083   0.1097
       4   3.038 BB    0.0420    7.50536    2.92268   0.0837
       5   3.285 BB    0.0435  196.45462   72.80782   2.1910
       6   3.406 BB    0.0436    9.71770    3.58381   0.1084
       7   3.630 BB    0.0529   13.10033    3.73795   0.1461

    Totals :                  8966.49877 2175.47659


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

Compound 5

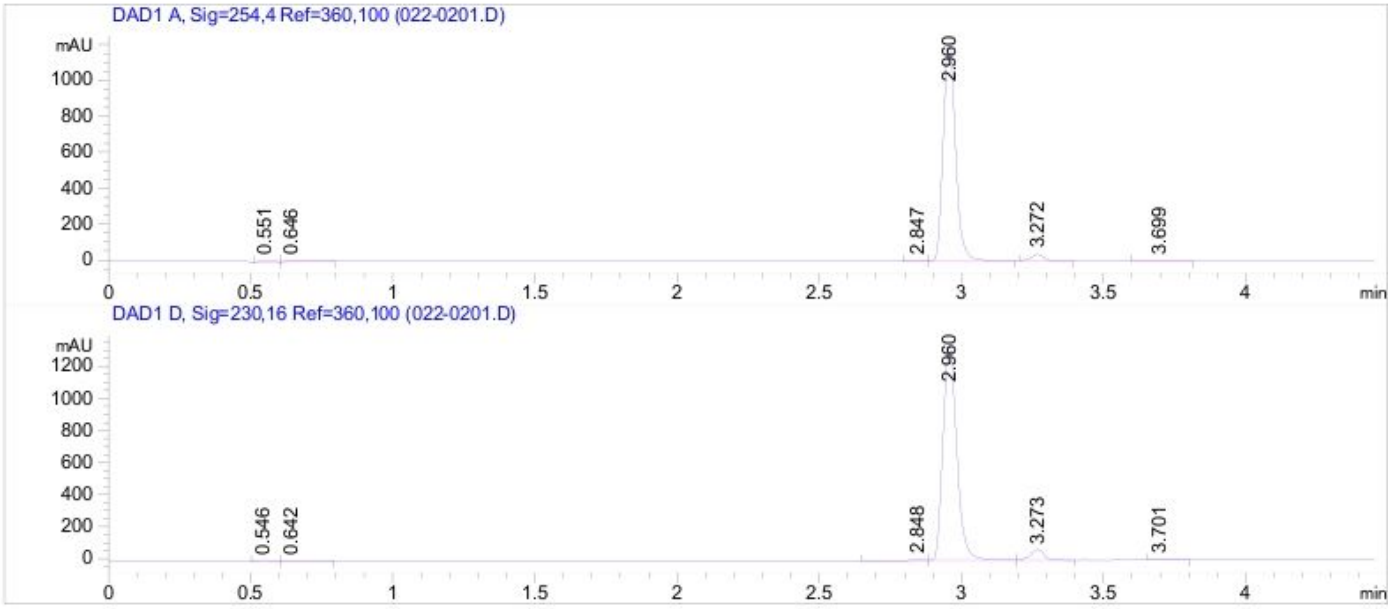

Compound 6
Normalized Intensity
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Compound 6
TOF MS ES+
1.15e3
100
%
0
311.05
352.12
312.30
374.31
621.30
643.14
375.34
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Sample Name: T-1650

```
    ==================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   2
    Acq. Instrument : Instrument 1                    Location : Vial 22
    Injection Date  : 1/9/2020 8:38:40 AM                 Inj :   1
                                                    Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M (
                      Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ==================================================================
```

Compound 6




```
    ==================================================================
                            Area Percent Report
    ==================================================================

    Sorted By             :      Signal
    Multiplier:                   :      1.0000
    Dilution:                     :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.551 BB    0.0438   21.60491    7.03463   0.5663
       2   0.646 BB    0.0603   17.42271    4.04187   0.4567
       3   2.847 BB    0.0391    8.06321    3.46912   0.2114
       4   2.960 BB    0.0472 3639.00562 1206.51001  95.3859
       5   3.272 BB    0.0499  117.67042   36.27000   3.0844
       6   3.699 BB    0.0531   11.26743    3.36524   0.2953

    Totals :                  3815.03429 1260.69087
```

Sample Name: T-1650

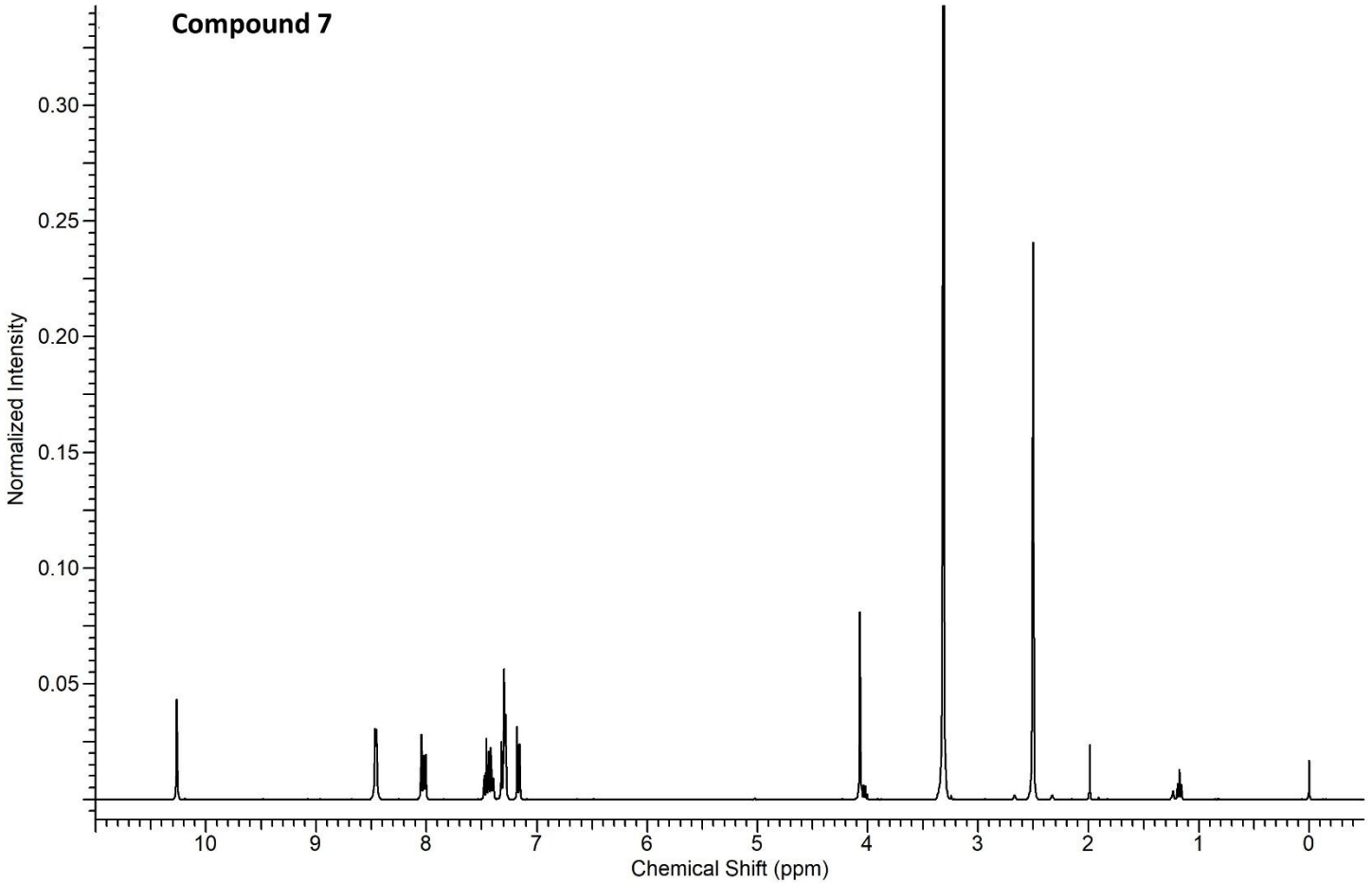
Acq. Operator : Tetra Discovery Partners Seq. Line : 2
Acq. Instrument : Instrument 1 Location : Vial 22
Injection Date : 1/9/2020 8:38:40 AM Inj : 1
Inj Volume : 5.0 µl
Sequence File : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-09 08-31-52\STD_SEQ.S
Method : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-09 08-31-52\STD_MTD.M (Sequence Method)
Last changed : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
Method Info : Standard method for small molecule separation

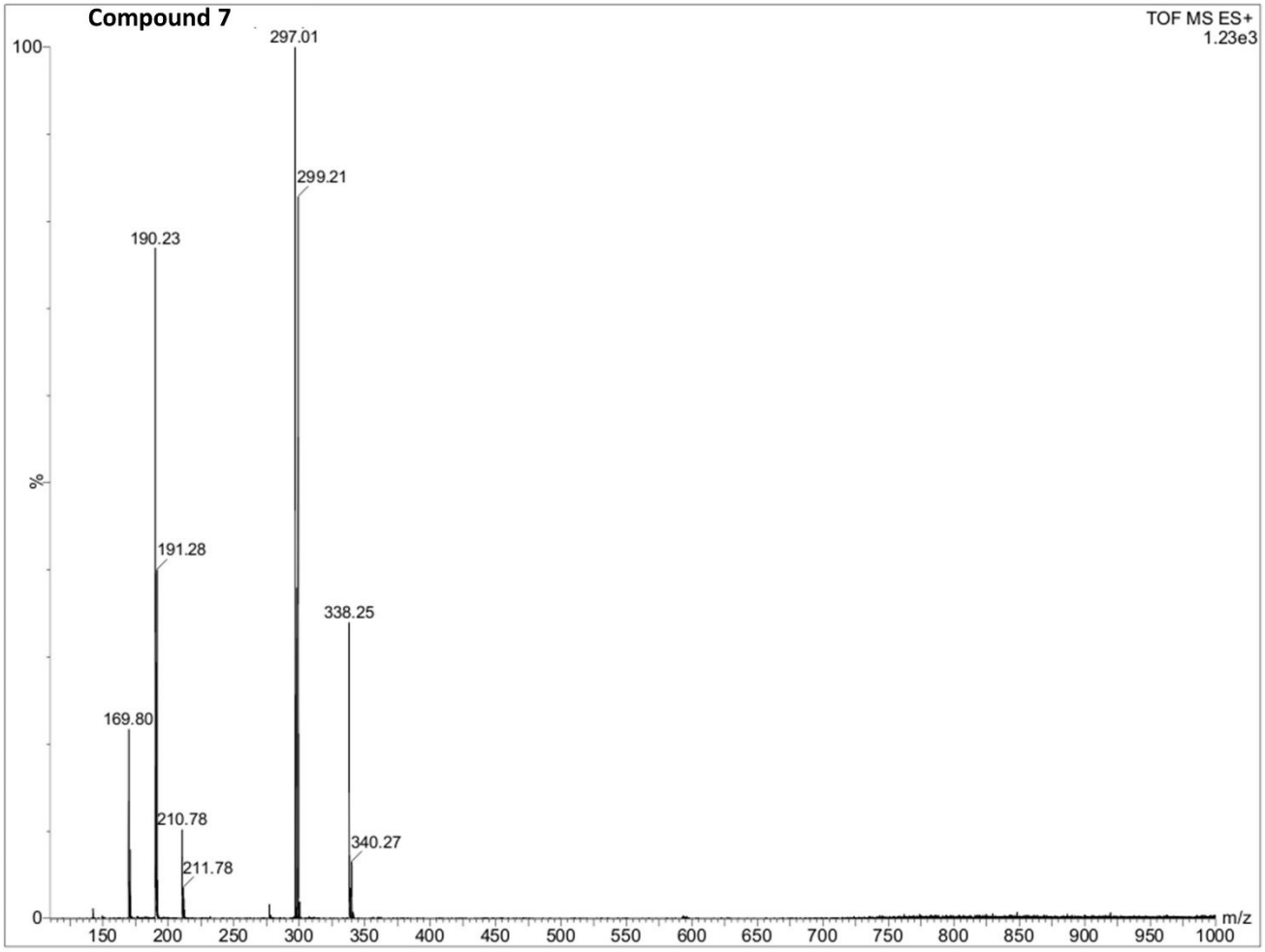
Compound 6

Signal 2: DAD1 D, Sig=230,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.546	BB	0.0411	27.30640	9.06798	0.5705
2	0.642	BB	0.0512	20.85920	5.63690	0.4358
3	2.848	BB	0.0300	9.02666	5.84961	0.1886
4	2.960	BB	0.0530	4497.88916	1343.84485	93.9666
5	3.273	BB	0.0499	215.06854	66.22860	4.4931
6	3.701	BB	0.0456	16.53905	5.73654	0.3455
Totals :				4786.68901	1436.36447	

*** End of Report ***

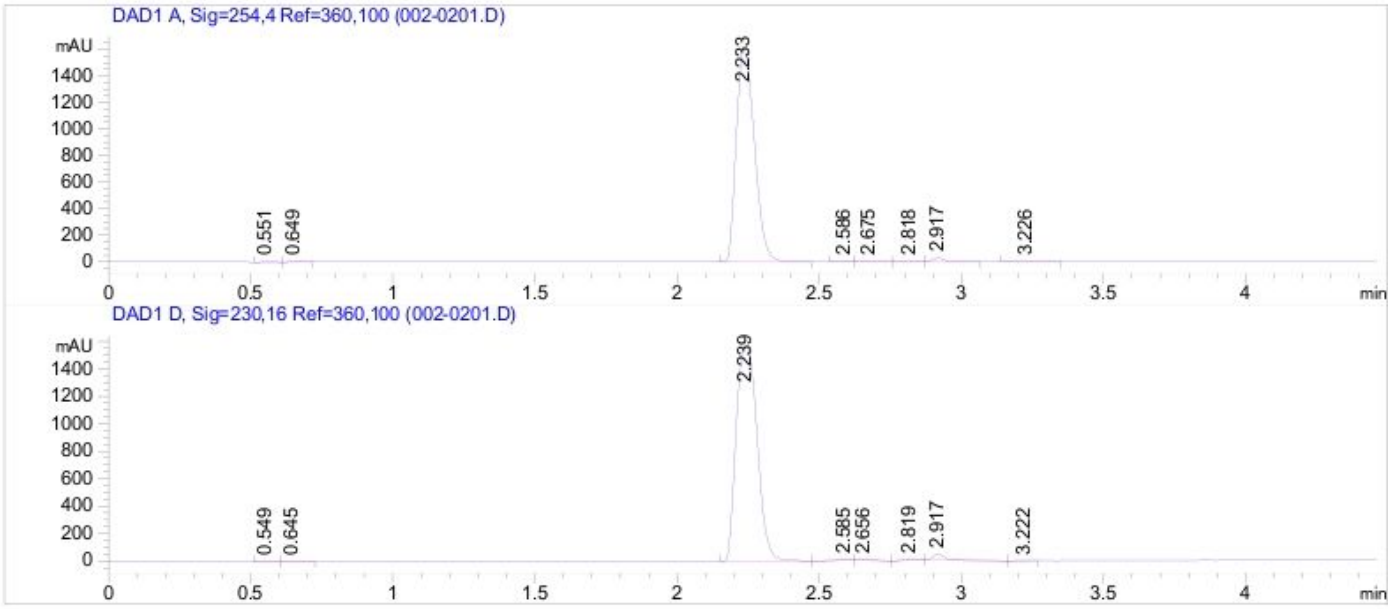

Compound 7
Normalized Intensity
0.30
0.25
0.20
0.15
0.10
0.05
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Compound 7
TOF MS ES+
1.23e3
100
%
0
297.01
299.21
190.23
191.28
338.25
169.80
210.78
211.78
340.27
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Sample Name: T-2009

```
==================================================================
Acq. Operator   : Tetra Discovery Partners       Seq. Line :   2
Acq. Instrument : Instrument 1                    Location : Vial 2
Injection Date  : 1/13/2020 5:09:16 PM                 Inj :   1
                                                 Inj Volume : 5.0 µl
Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-13 17-01-50\STD_SEQ.S
Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-13 17-01-50\STD_MTD.M (
                  Sequence Method)
Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
Method Info     : Standard method for small molecule separation

==================================================================
```

Compound 7




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.551 BB    0.0440   20.79967    6.73318   0.2871
   2   0.649 BB    0.0384    8.32270    3.19299   0.1149
   3   2.233 BB    0.0714 7125.55713 1618.07104  98.3542
   4   2.586 BB    0.0373    6.01464    2.57073   0.0830
   5   2.675 BB    0.0788    7.19062    1.53099   0.0993
   6   2.818 BB    0.0505    8.99566    2.72732   0.1242
   7   2.917 BB    0.0405   61.47284   25.19331   0.8485
   8   3.226 BB    0.0666    6.43829    1.42934   0.0889

Totals :                  7244.79156 1661.44890
```

Sample Name: T-2009

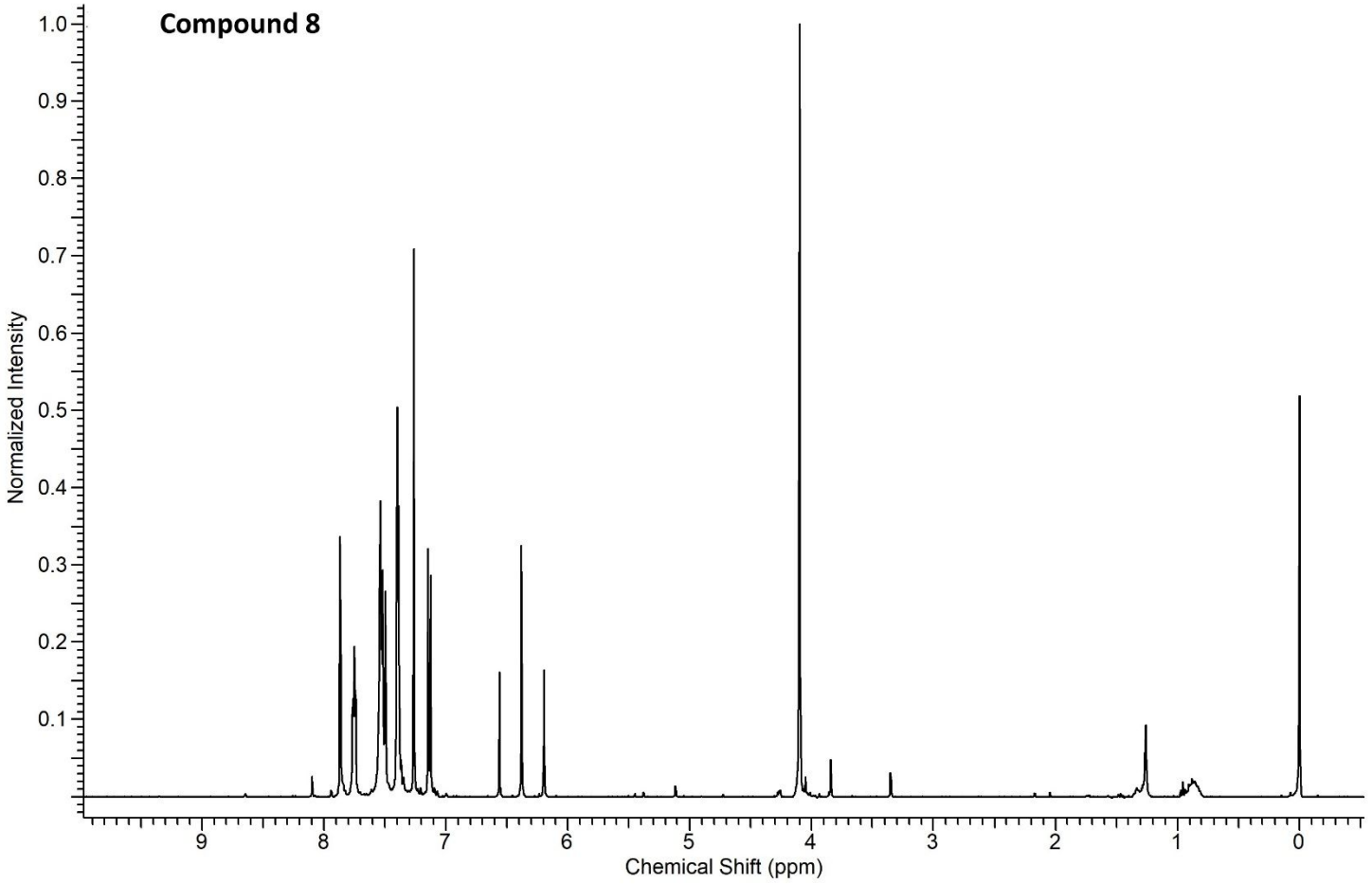
Acq. Operator : Tetra Discovery Partners Seq. Line : 2
Acq. Instrument : Instrument 1 Location : Vial 2
Injection Date : 1/13/2020 5:09:16 PM Inj : 1
Inj Volume : 5.0 µl
Sequence File : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-13 17-01-50\STD_SEQ.S
Method : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-13 17-01-50\STD_MTD.M (Sequence Method)
Last changed : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
Method Info : Standard method for small molecule separation

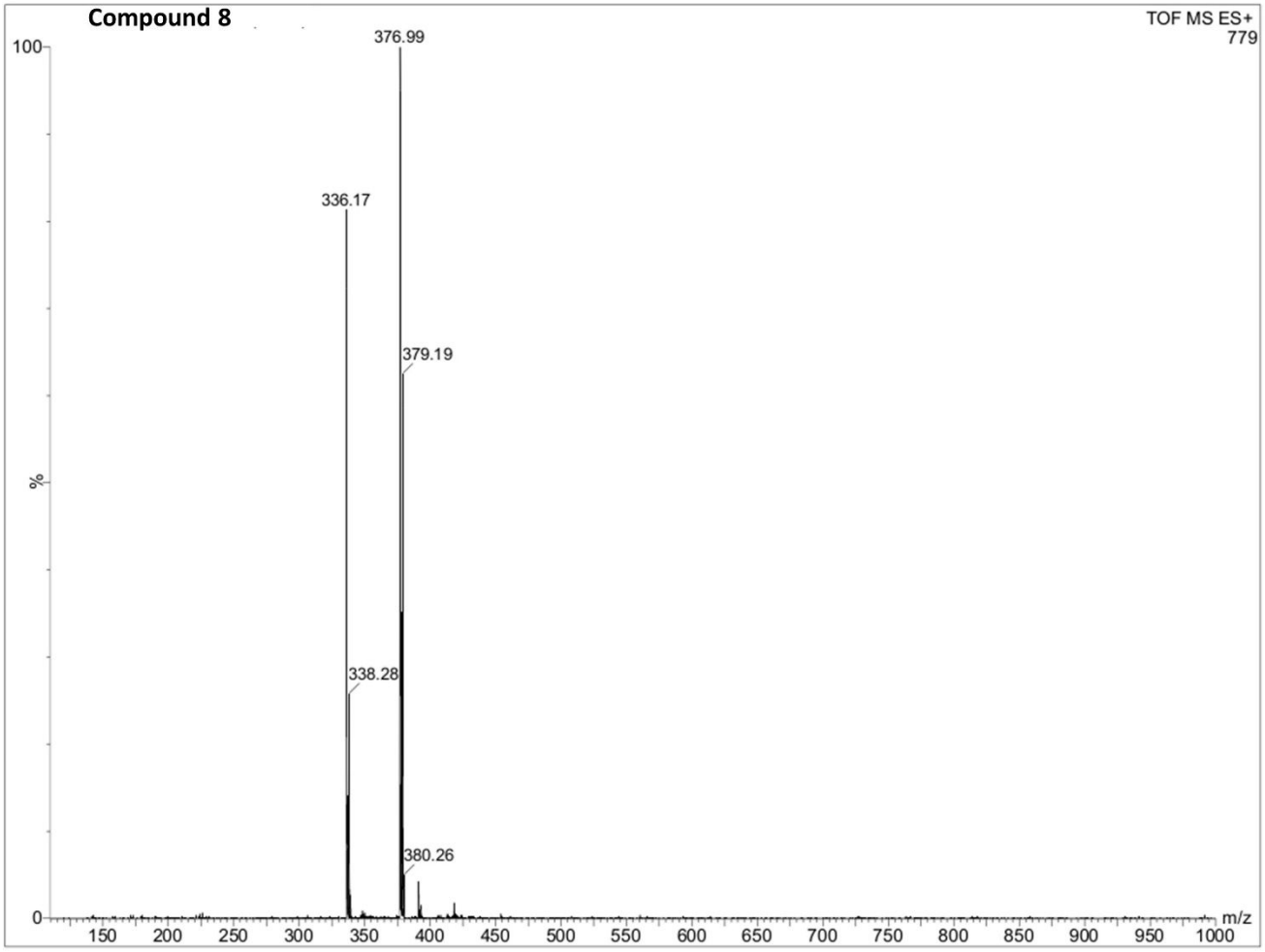
Compound 7

Signal 2: DAD1 D, Sig=230,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.549	BB	0.0408	24.67207	8.26218	0.3006
2	0.645	BB	0.0390	10.77763	4.05573	0.1313
3	2.239	BB	0.0839	8027.73975	1563.24561	97.7997
4	2.585	BB	0.0402	11.27485	4.67100	0.1374
5	2.656	BB	0.0860	8.81802	1.66064	0.1074
6	2.819	BB	0.0521	15.90209	4.62795	0.1937
7	2.917	BB	0.0405	104.07726	42.66168	1.2679
8	3.222	BB	0.0480	5.08581	1.74383	0.0620
Totals :				8208.34748	1630.92862	

*** End of Report ***

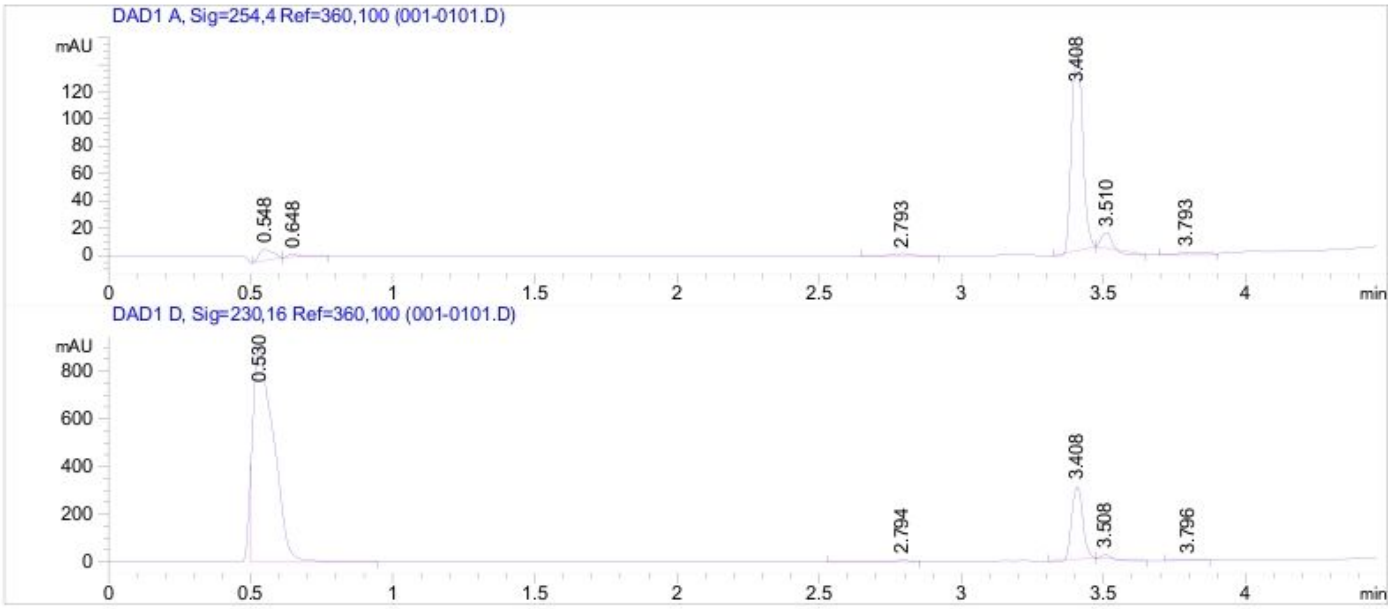

Compound 8
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Compound 8
TOF MS ES+
779
100
%
0
376.99
336.17
379.19
338.28
380.26
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Sample Name: T-2517

```
================================================================================
Acq. Operator   : Tetra Discovery Partners       Seq. Line :   1
Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 1/13/2020 4:25:25 PM                Inj :   1
                                                 Inj Volume : 5.0 µl
Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-13 16-24-33\STD_SEQ.S
Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-13 16-24-33\STD_MTD.M (
                  Sequence Method)
Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
Method Info     : Standard method for small molecule separation

================================================================================
```

Compound 8

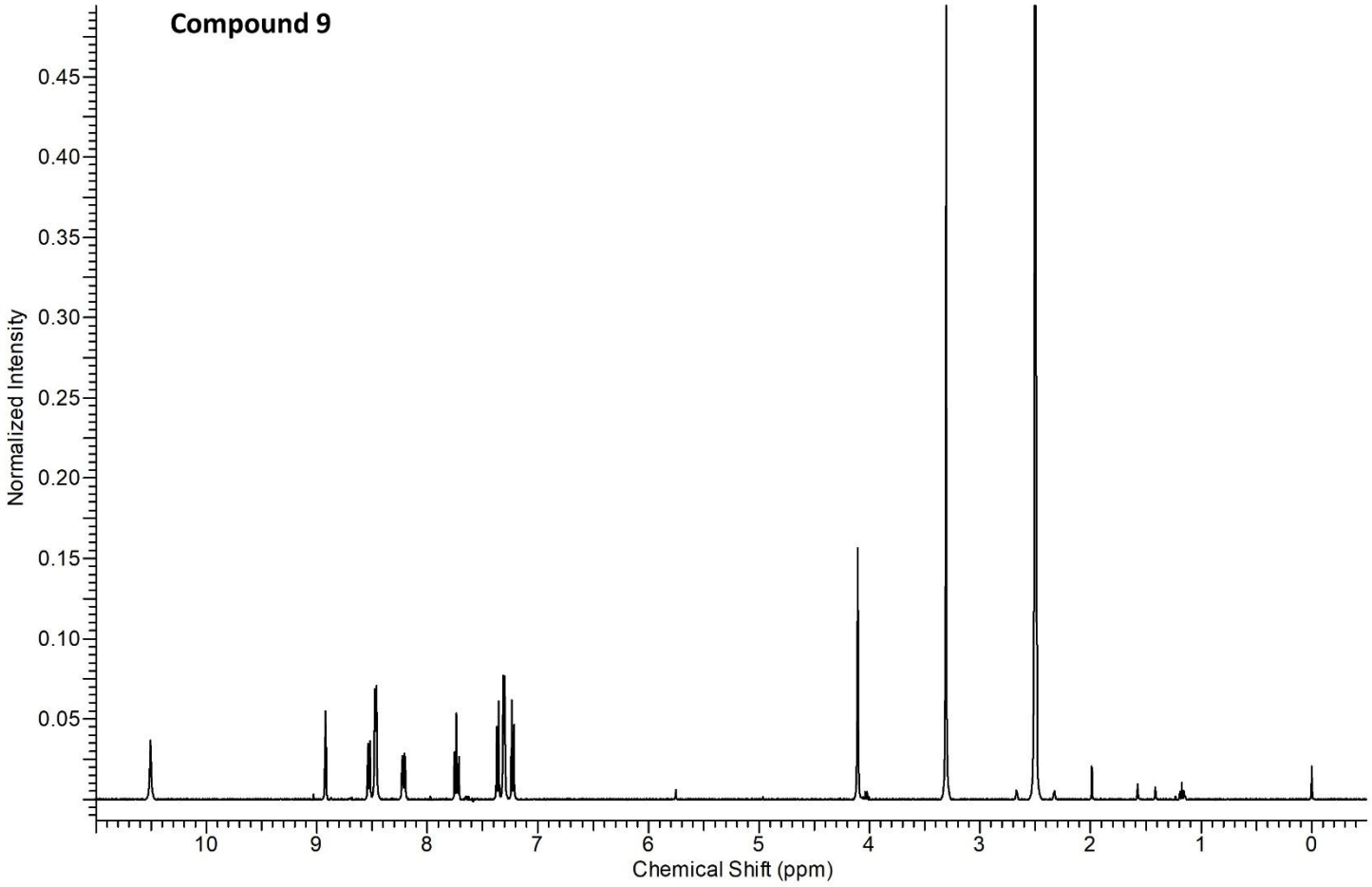


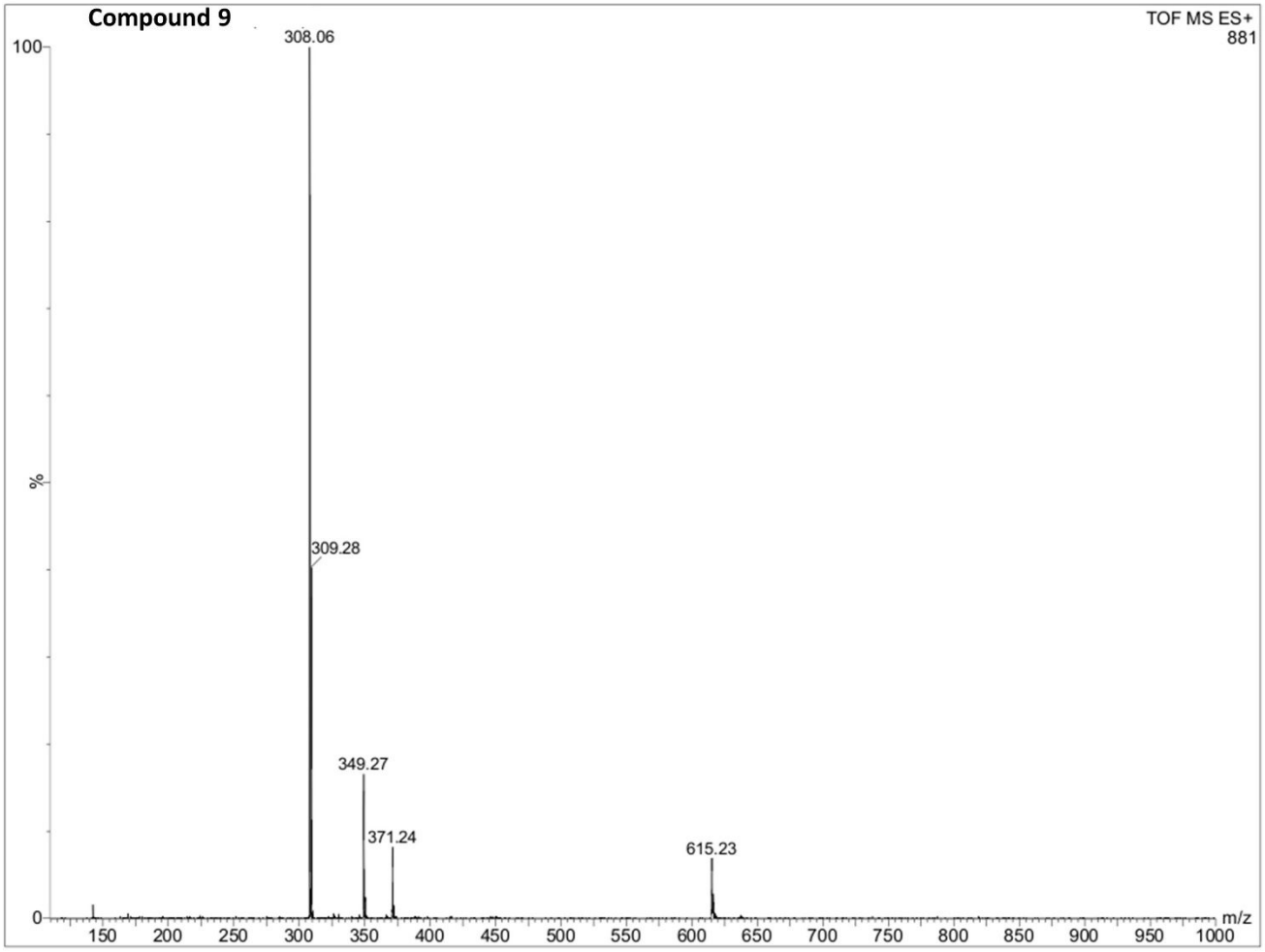

```
================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.548 BB    0.0464   27.47399    8.35339   5.8340
   2   0.648 BB    0.0414    7.44672    2.45165   1.5813
   3   2.793 BB    0.0709    9.88186    1.95694   2.0984
   4   3.408 BB    0.0432  398.36746  149.02811  84.5920
   5   3.510 BB    0.0336   21.82435   11.79237   4.6343
   6   3.793 BB    0.0545    5.93382    1.62932   1.2600

Totals :                   470.92820  175.21178
```

Sample Name: T-2517

Compound 8

```
    ==================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   1
    Acq. Instrument : Instrument 1                    Location : Vial 1
    Injection Date  : 1/13/2020 4:25:25 PM                 Inj :   1
                                                     Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-13 16-24-33\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-13 16-24-33\STD_MTD.M (
                      Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ==================================================================


    Signal 2: DAD1 D, Sig=230,16 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.530 BB    0.0693 4468.31348  909.69635  83.9057
       2   2.794 BB    0.0489   15.79040    4.99276   0.2965
       3   3.408 BB    0.0432  820.19733  306.69583  15.4016
       4   3.508 BB    0.0235   15.11948   15.29594   0.2839
       5   3.796 BB    0.0561    5.97869    1.58137   0.1123

    Totals :                  5325.39938 1238.26225


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

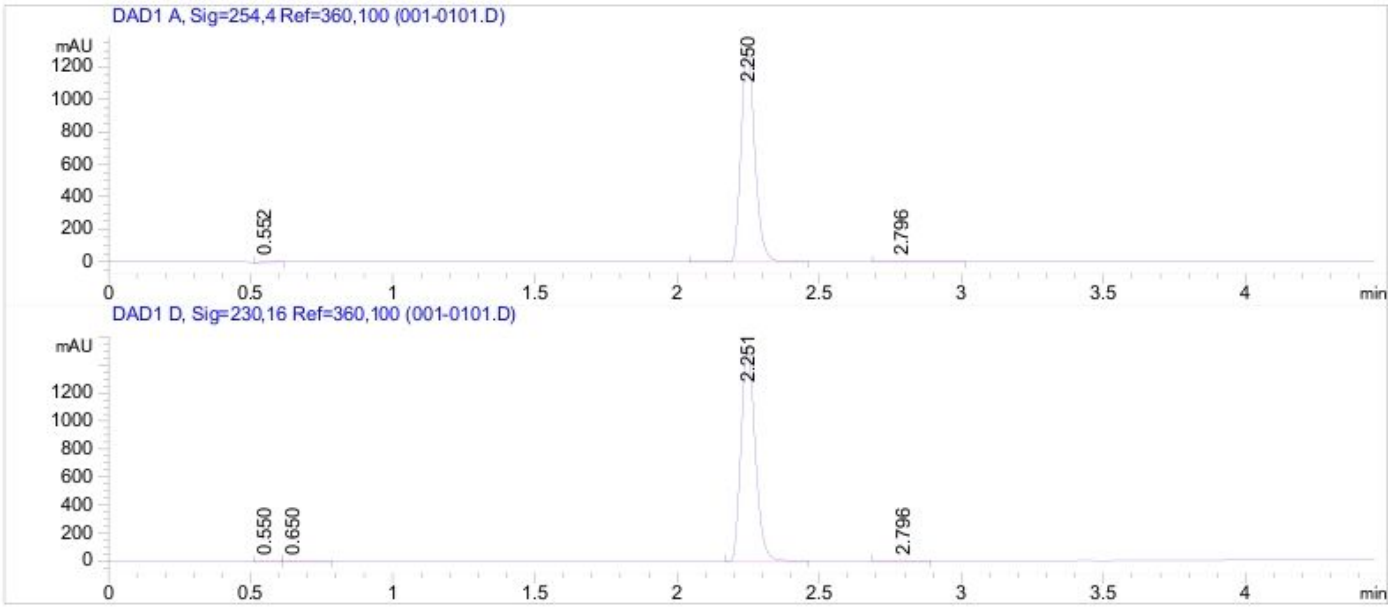

Compound 9
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


Compound 9
TOF MS ES+
881
100
%
0
308.06
309.28
349.27
371.24
615.23
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

Sample Name: T-1842

```
    ========================================================================
    Acq. Operator    : Tetra Discovery Partners       Seq. Line :   1
    Acq. Instrument  : Instrument 1                    Location : Vial 1
    Injection Date   : 1/13/2020 5:03:09 PM                Inj :   1
                                                     Inj Volume : 5.0 µl
    Sequence File    : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-13 17-01-50\STD_SEQ.S
    Method           : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-13 17-01-50\STD_MTD.M (
                       Sequence Method)
    Last changed     : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info      : Standard method for small molecule separation

    ========================================================================
```

Compound 9



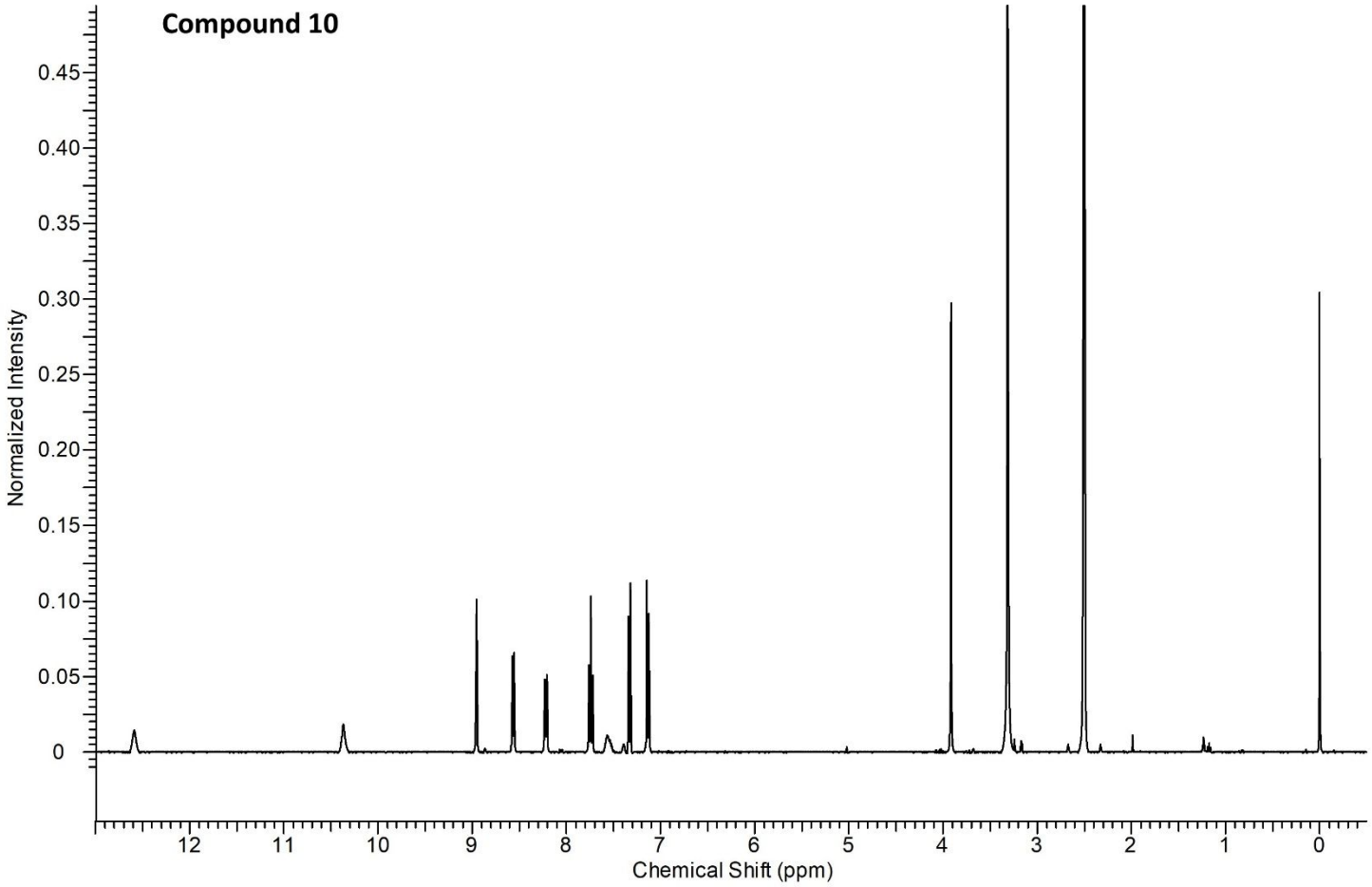

```
    ========================================================================
                              Area Percent Report
    ========================================================================

    Sorted By             :      Signal
    Multiplier:                   :      1.0000
    Dilution:                     :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.552 BB    0.0443   19.45042    6.25359   0.4405
       2   2.250 BB    0.0527 4386.11084 1320.84546  99.3406
       3   2.796 BB    0.0737    9.66268    1.82679   0.2188

    Totals :                  4415.22394 1328.92584
```

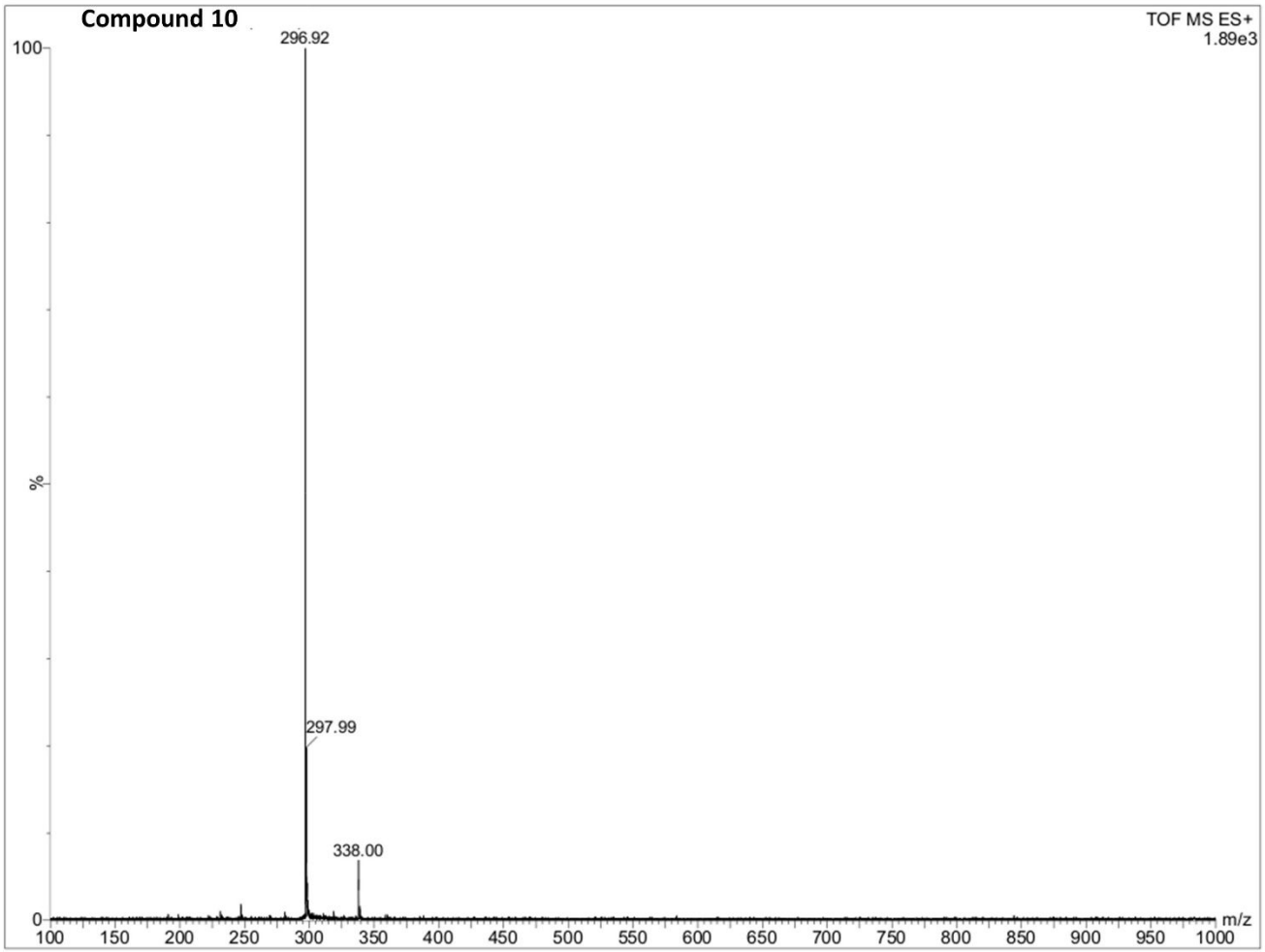
Sample Name: T-1842

```
    ======================================================================
    Acq. Operator   : Tetra Discovery Partners       Seq. Line :   1
    Acq. Instrument : Instrument 1                    Location : Vial 1
    Injection Date  : 1/13/2020 5:03:09 PM                 Inj :   1
                                                    Inj Volume : 5.0 µl
    Sequence File   : C:\Users\Public\Documents\Agilent1100\STD_SEQ 2020-01-13 17-01-50\STD_SEQ.S
    Method          : C:\USERS\PUBLIC\DOCUMENTS\AGILENT1100\STD_SEQ 2020-01-13 17-01-50\STD_MTD.M (
                      Sequence Method)
    Last changed    : 10/17/2019 4:57:20 PM by Tetra Discovery Partners
    Method Info     : Standard method for small molecule separation

    ======================================================================
```

Compound 9

```
    Signal 2: DAD1 D, Sig=230,16 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   0.550 BB    0.0438   24.93855    8.13513   0.4693
       2   0.650 BB    0.0427    9.65048    3.24490   0.1816
       3   2.251 BB    0.0541 5273.07471 1532.46899  99.2205
       4   2.796 BB    0.0576    6.83724    1.74887   0.1287

    Totals :                   5314.50097 1545.59790

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

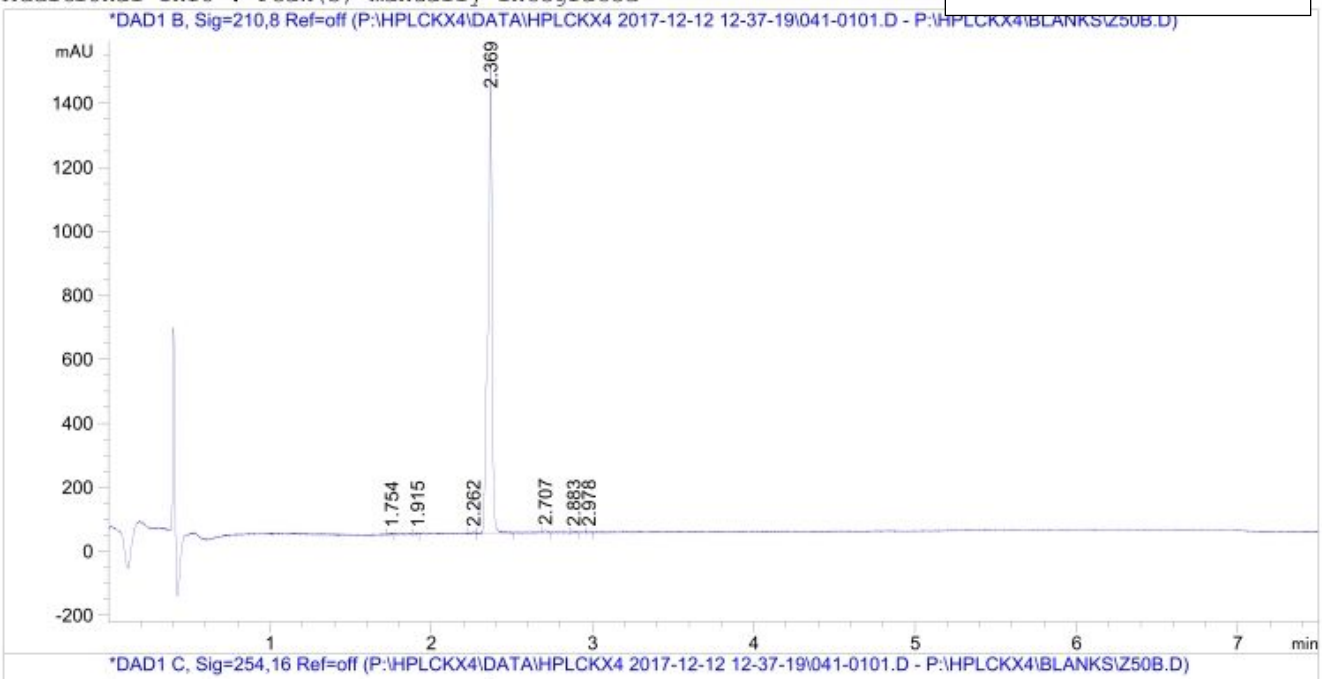

Compound 10
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

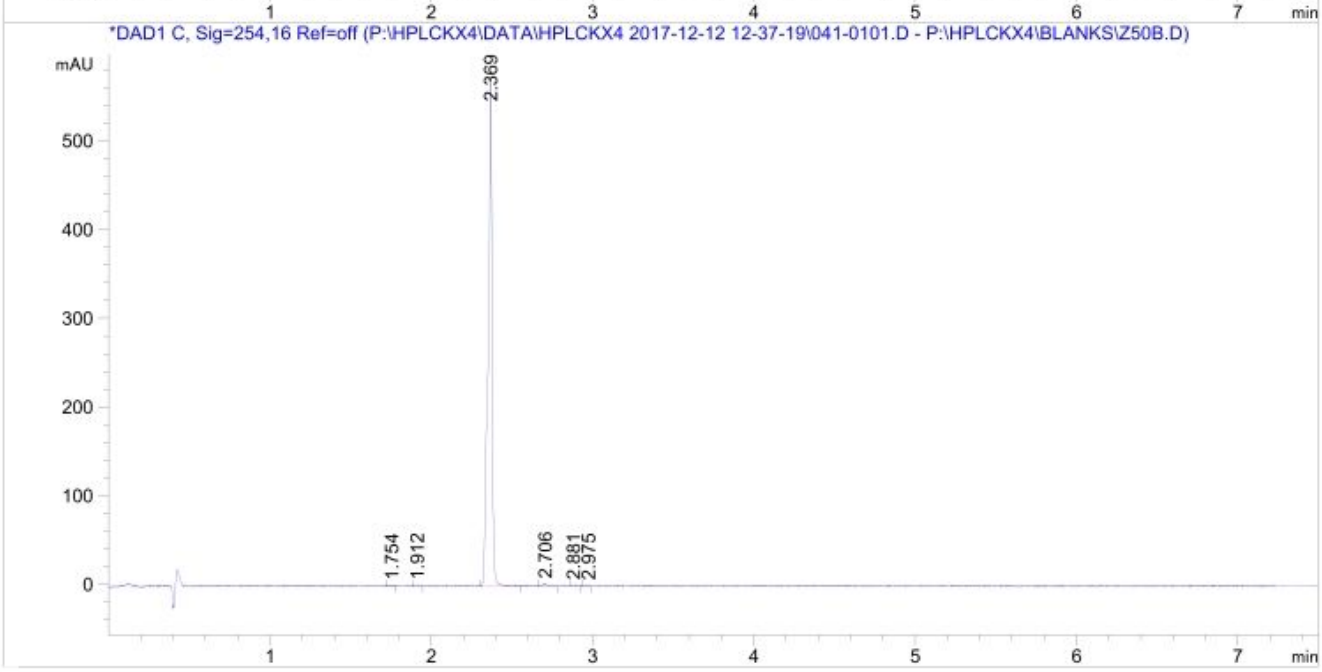

Compound 10
TOF MS ES+
1.89e3
296.92
297.99
338.00
100
%
0
m/z
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000

Sample Name: T-1720 140-57-011

```
==================================================================
Acq. Operator   :                                Seq. Line :   1
Acq. Instrument : Instrument 1                    Location : Vial 41
Injection Date  : 12/12/2017 12:38:43 PM               Inj :   1
                                                 Inj Volume : 5.0 µl
Acq. Method     : P:\HPLCKX4\DATA\HPLCKX4 2017-12-12 12-37-19\Z50.M
Last changed    : 9/6/2017 12:28:06 PM
Analysis Method : C:\CHEM32\1\METHODS\DEF_LC.M
Last changed    : 12/13/2017 2:11:35 PM
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

Compound 10





Sample Name: T-1720 140-57-011

Compound 10

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=210,8 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.754	BV	0.0142	7.35588	6.50598	0.2876
2	1.915	BB	0.0123	5.81374	6.40295	0.2273
3	2.262	BV	0.0195	12.87690	7.89297	0.5034
4	2.369	VB	0.0247	2499.27222	1441.28113	97.6996
5	2.707	BB	0.0158	10.80134	8.67032	0.4222
6	2.883	BV	0.0265	13.45817	6.81933	0.5261
7	2.978	BB	0.0223	8.54099	5.15849	0.3339
Totals :				2558.11924	1482.73116	

Signal 2: DAD1 C, Sig=254,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.754	BB	0.0196	2.37219	1.49159	0.2413
2	1.912	BB	0.0203	3.18693	1.98156	0.3242
3	2.369	BB	0.0235	966.31439	570.21277	98.3069
4	2.706	BB	0.0236	5.94882	3.10076	0.6052
5	2.881	BB	0.0228	3.38616	1.80849	0.3445
6	2.975	BB	0.0159	1.74863	1.37248	0.1779
Totals :				982.95712	579.96765	

*** End of Report ***