Supplementary Information

Investigation of the fuzzy complex between RSV nucleoprotein and phosphoprotein to optimize an inhibition assay by fluorescence polarization

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UPLC Traces

Sample name: **P₃**

Solvent A: H₂O+0.1%HCOOH

Column: BEH C18 2.1x50 mm 1.7 µm

Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S7. UPLC analysis of **P₃**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **P₄**

Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H₂O+0.1%HCOOH

Solvent B: Acetonitrile +0,1% HCOOH

Figure S8. UPLC analysis of **P₄**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **P₅ₑ** Sample name: **P$_{5E}$**

Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

g)

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S9. UPLC analysis of **P$_{5E}$**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI$^+$), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **P7E**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
g)	Initial	0.600	95.0	5.0	Initial
	0.10	0.600	95.0	5.0	6
	0.20	0.600	80.0	20.0	6
	2.50	0.600	70.0	30.0	6
	2.55	0.600	0.0	100.0	6
	3.0	0.600	0.0	100.0	6
	3.10	0.600	95.0	5.0	6
	4.00	0.600	95.0	5.0	6

Figure S10. UPLC analysis of **P7E**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI+), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **P7E**
Column: BEH C18 2.1x50 mm 1.7 μm

Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

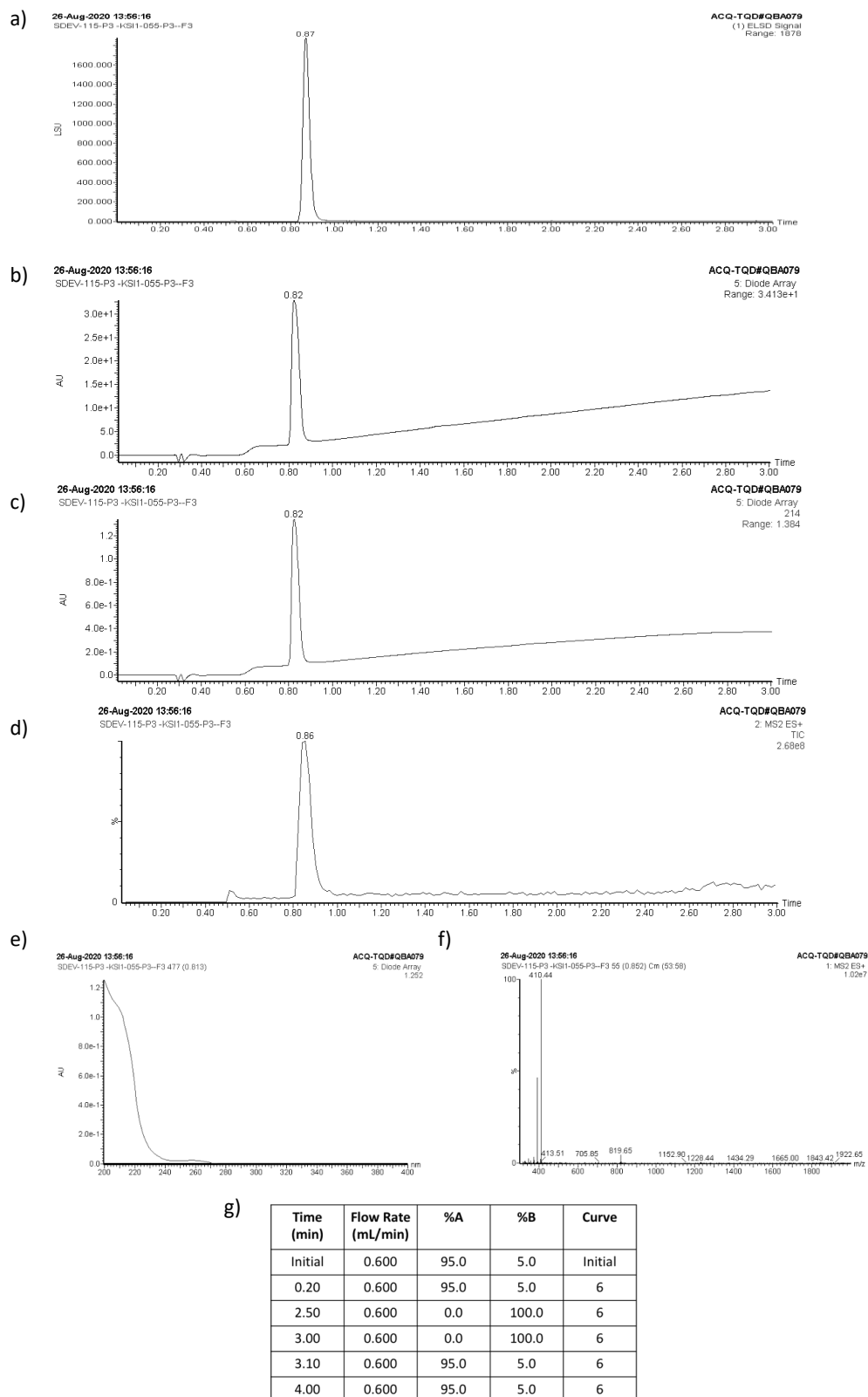| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.10	0.600	95.0	5.0	6
0.20	0.600	80.0	20.0	6
2.50	0.600	70.0	30.0	6
2.55	0.600	0.0	100.0	6
3.0	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S11. UPLC analysis of **P9E**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **P₁₁ᴇ**

Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H₂O+0.1%HCOOH
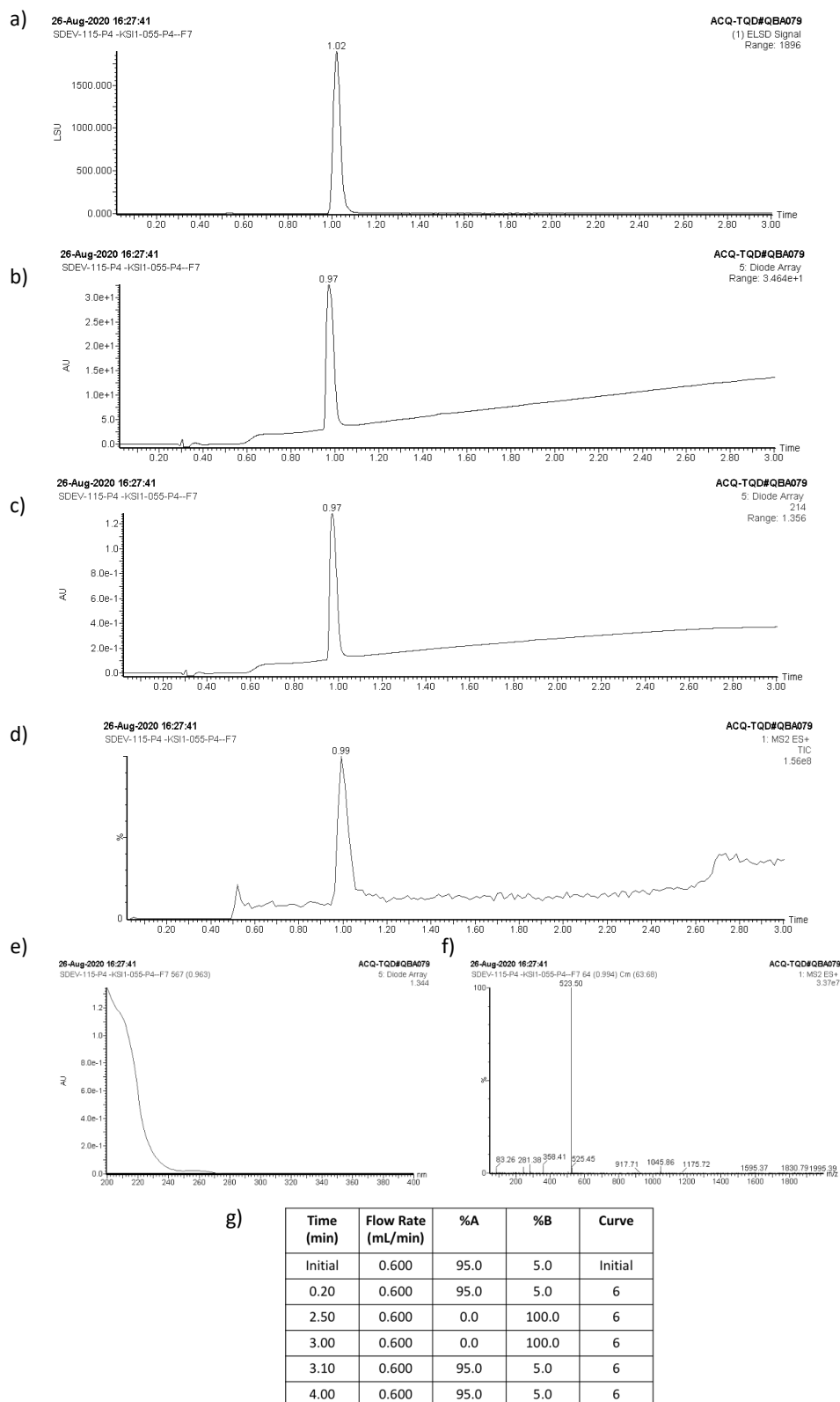
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

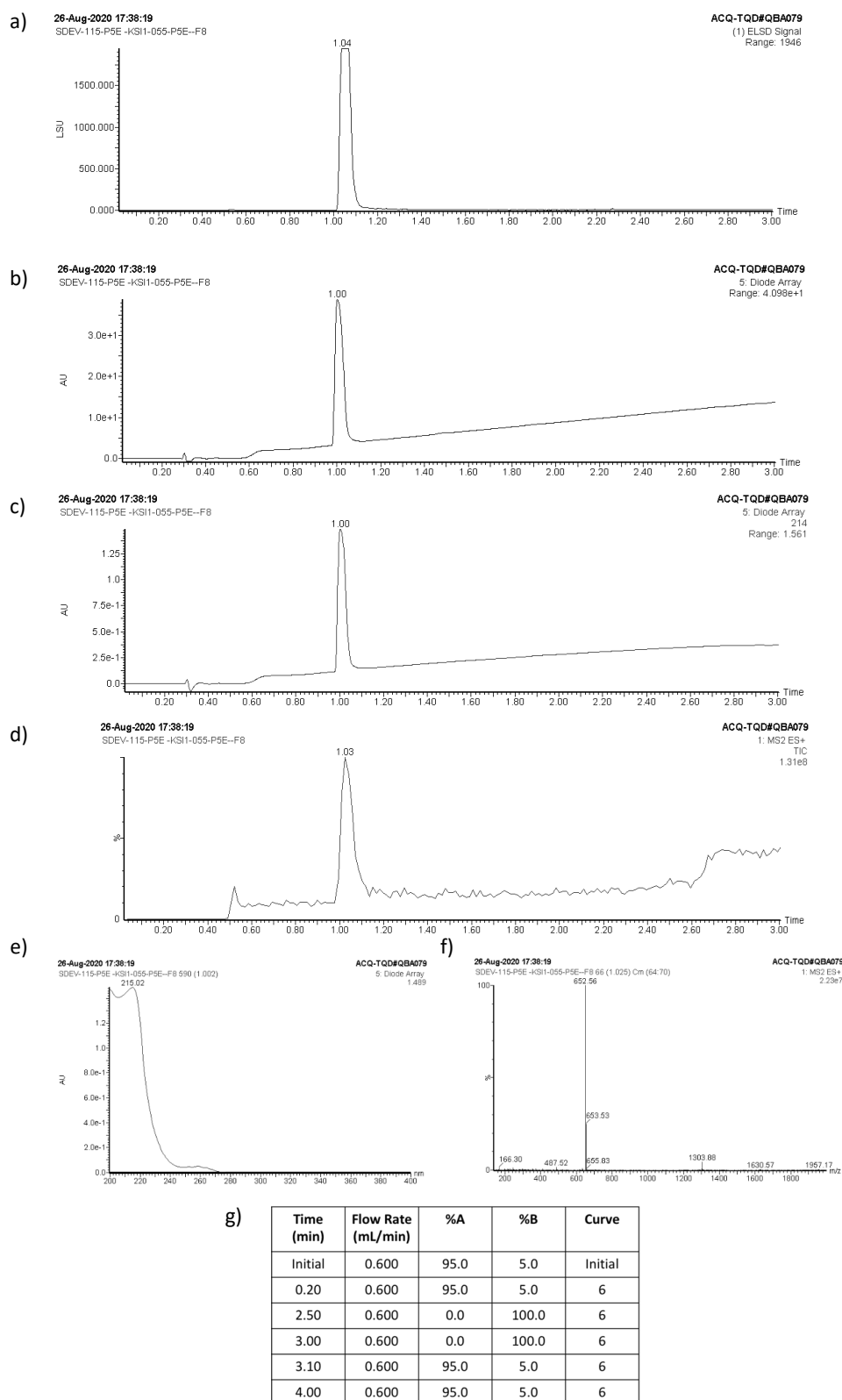| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.10	0.600	95.0	5.0	6
0.20	0.600	80.0	20.0	6
2.50	0.600	70.0	30.0	6
2.55	0.600	0.0	100.0	6
3.0	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S12. UPLC analysis of **P₁₁ᴇ**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

S11

Sample name: **F₃**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

01-Sep-2020 12:54:58
SDE01-117-KSI1-056-F3-grad25-45-F10

ACQ-TQD#QBA079
(1) ELSD Signal
Range: 181

b)

01-Sep-2020 12:54:58
SDE01-117-KSI1-056-F3-grad25-45-F10

ACQ-TQD#QBA079
5: Diode Array
Range: 9.091e+1

c)

01-Sep-2020 12:54:58
SDE01-117-KSI1-056-F3-grad25-45-F10

ACQ-TQD#QBA079
5: Diode Array
214
Range: 1.646

d)

01-Sep-2020 12:54:58
SDE01-117-KSI1-056-F3-grad25-45-F10

ACQ-TQD#QBA079
4: MS2 ES-
TIC
9.48e6

e)

01-Sep-2020 12:54:58
SDE01-117-KSI1-056-F3-grad25-45-F10 1537 (1.300)

ACQ-TQD#QBA079
5: Diode Array
1.125

f)

01-Sep-2020 12:54:58
SDE01-117-KSI1-056-F3-grad25-45-F10 85 (1.332) Cm (84:87)

ACQ-TQD#QBA079
3: MS2 ES-
4.40e5

g)

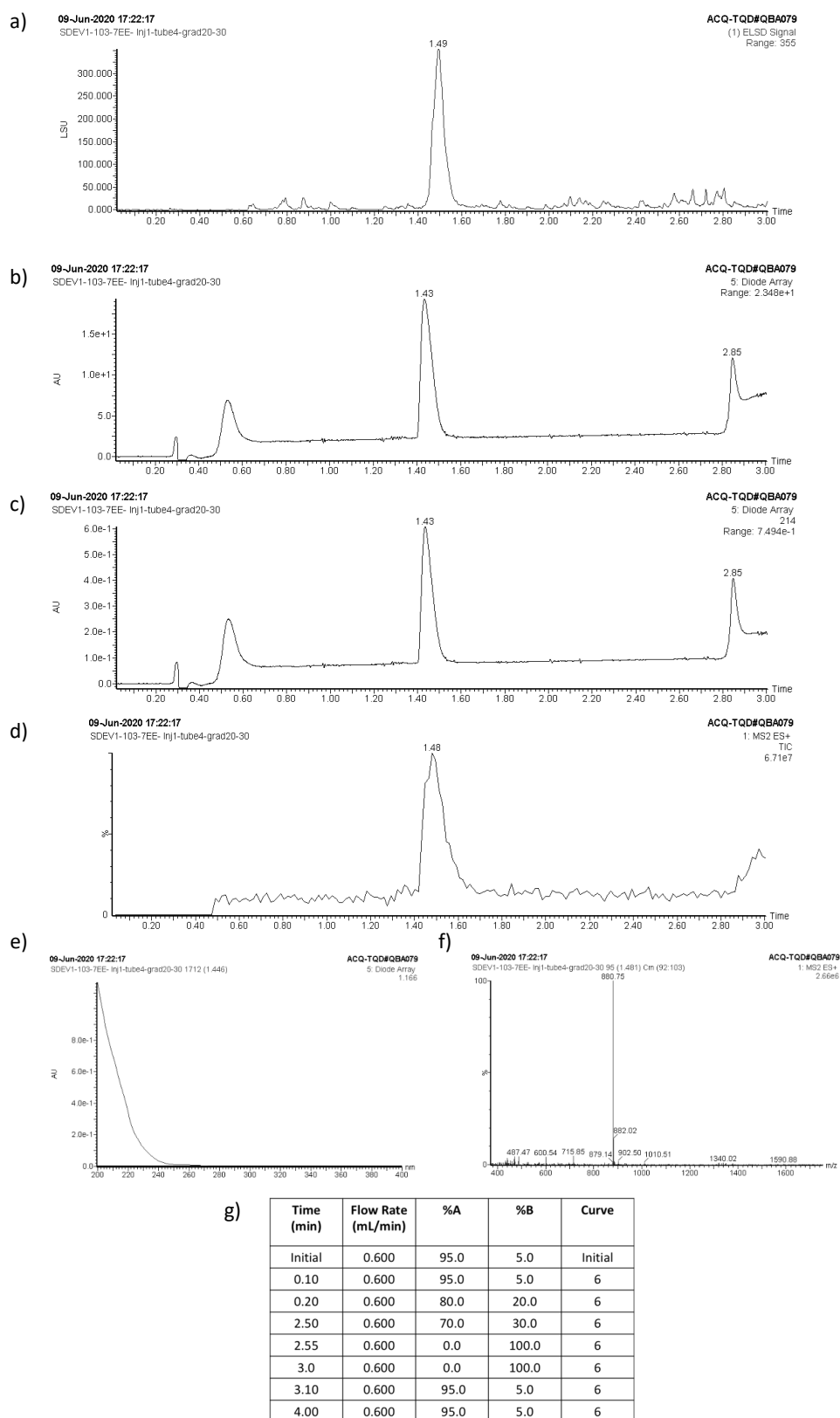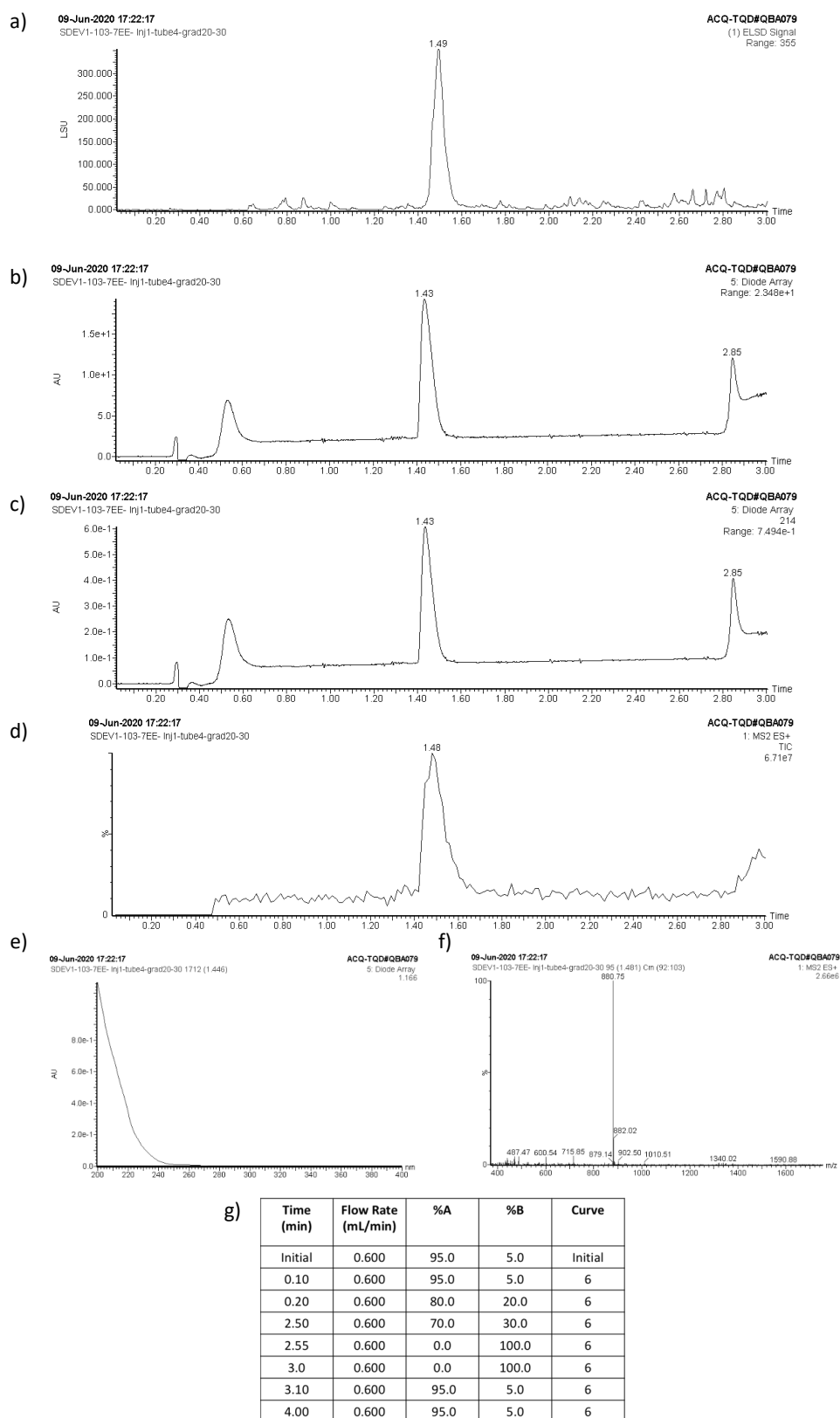| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.10	0.600	95.0	5.0	6
0.20	0.600	75.0	25.0	6
2.50	0.600	55.0	45.0	6
2.55	0.600	0.0	100.0	6
3.0	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S13. UPLC analysis of **F₃**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F4**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H2O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

g)

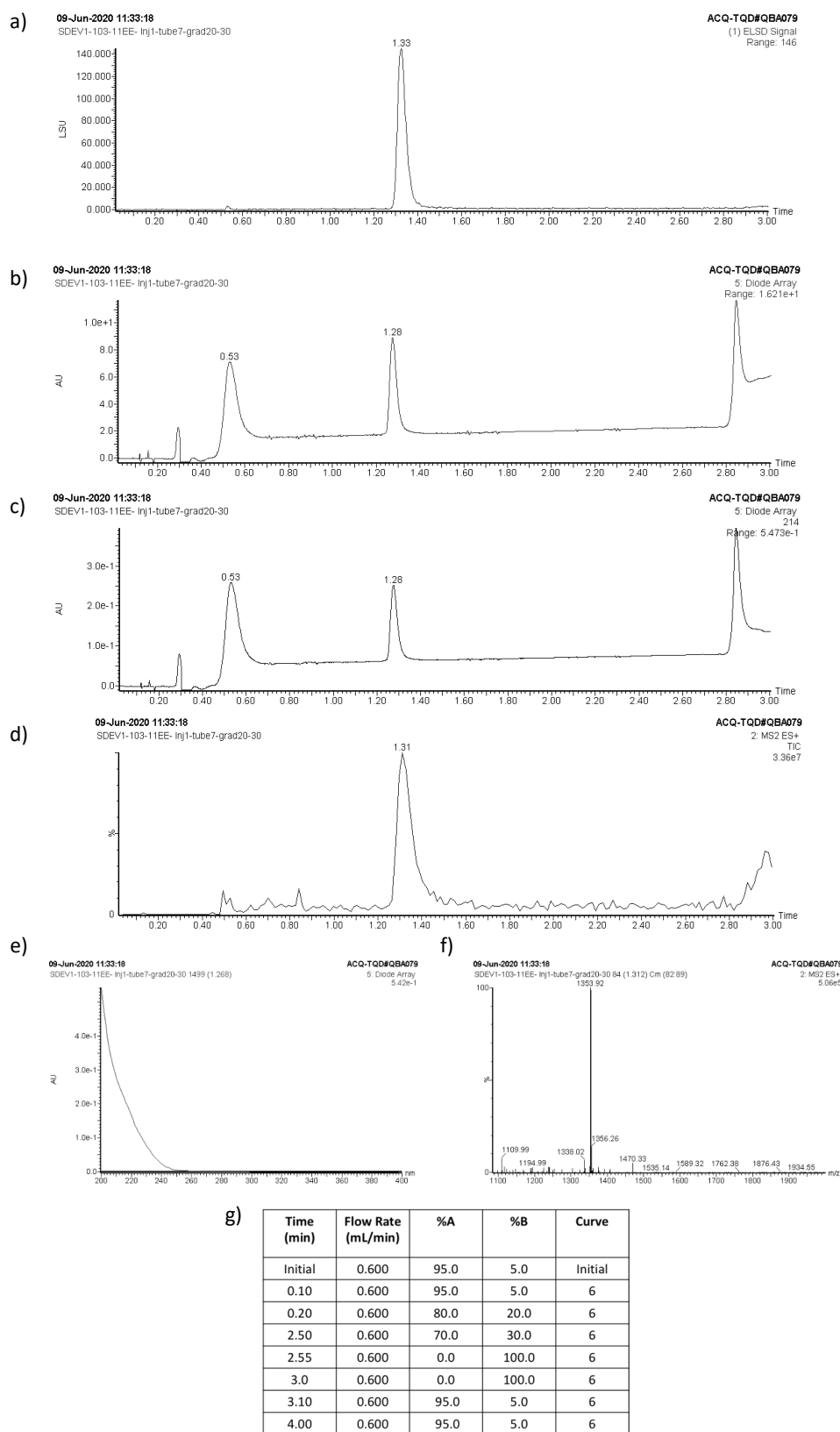| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S14. UPLC analysis of **F4**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F₅ₑ**
Column: BEH C18 2.1x50 mm 1.7 µm

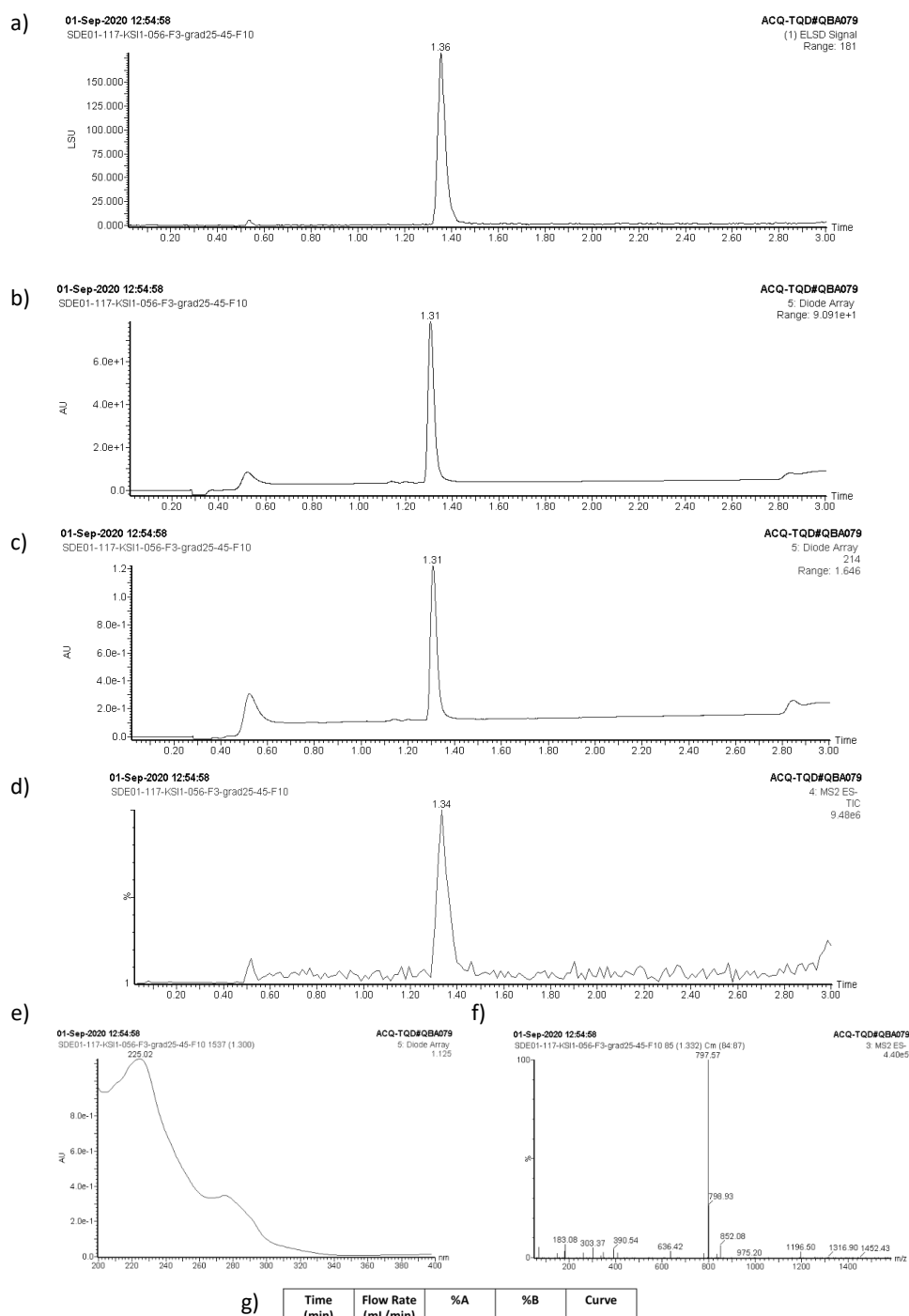Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.10	0.600	95.0	5.0	6
0.20	0.600	65.0	35.0	6
2.50	0.600	55.0	45.0	6
2.55	0.600	0.0	100.0	6
3.0	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S15. UPLC analysis of **F₅ₑ**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F₇E**
Column: BEH C18 2.1x50 mm 1.7 µm

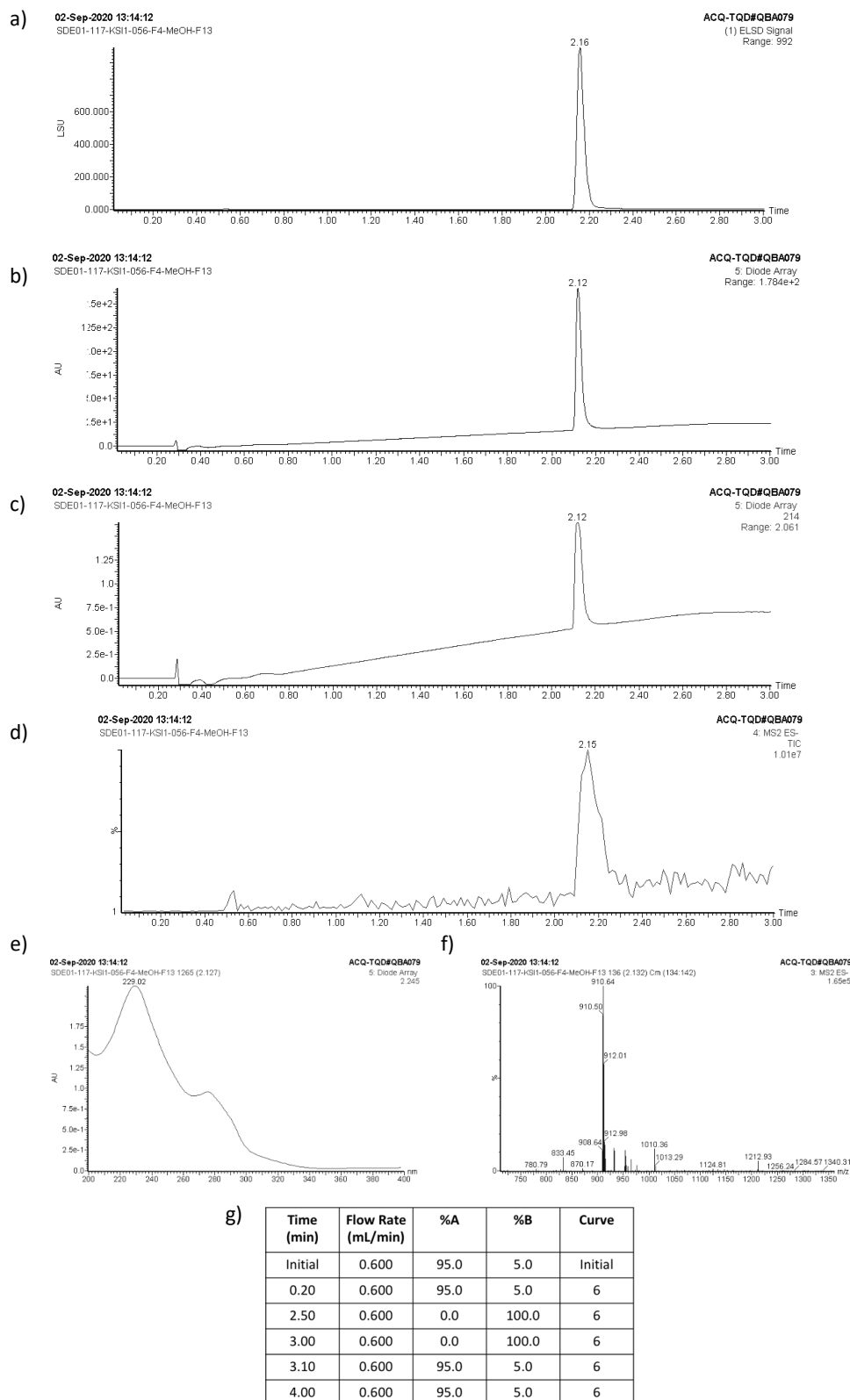Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a) ELSD Signal
b) Diode Array
c) Diode Array (214)
d) MS2 ES-TIC
e) absorption spectra
f) Mass spectra

g)

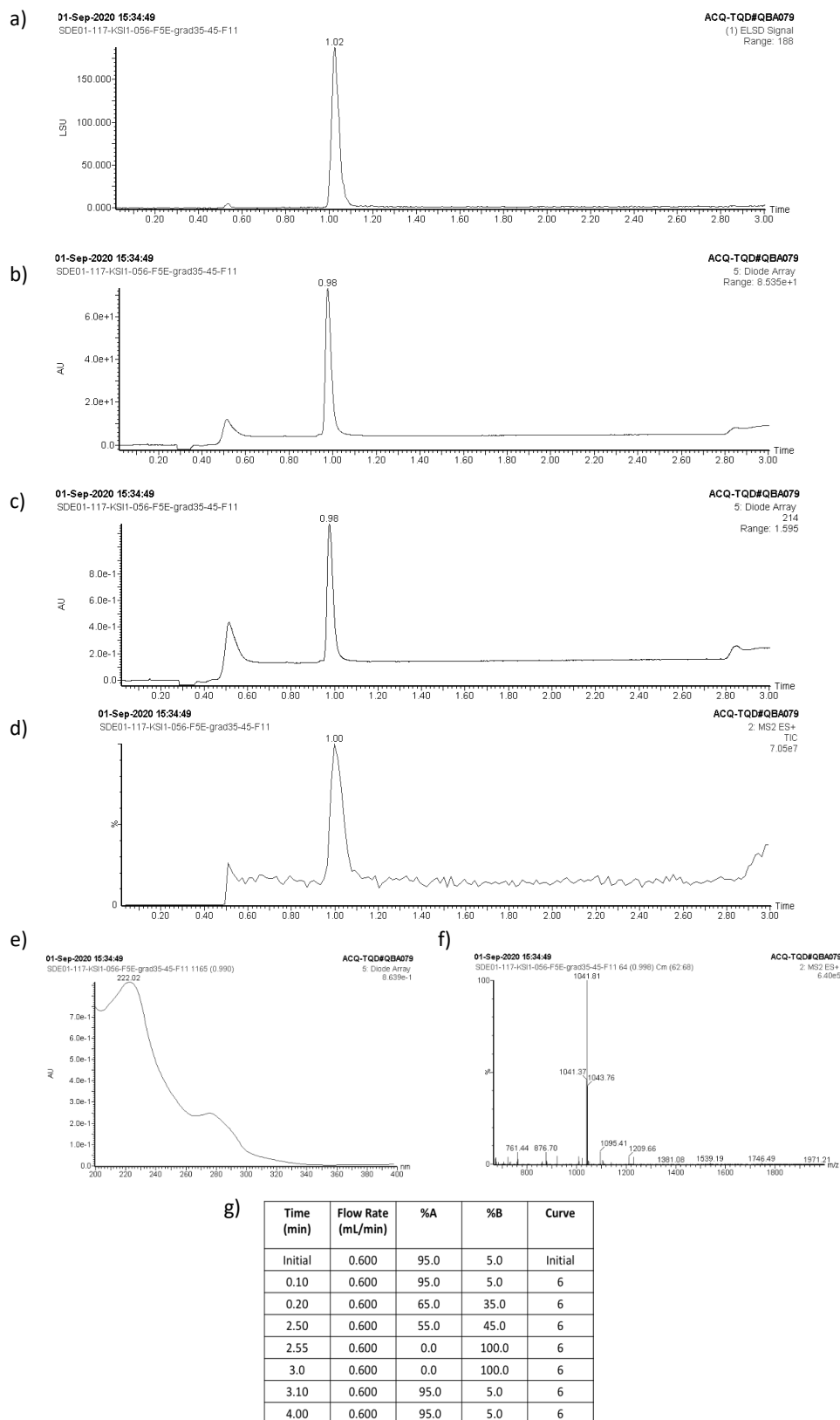| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S16. UPLC analysis of **F₇E**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F9E**
Column: BEH C18 2.1x50 mm 1.7 µm

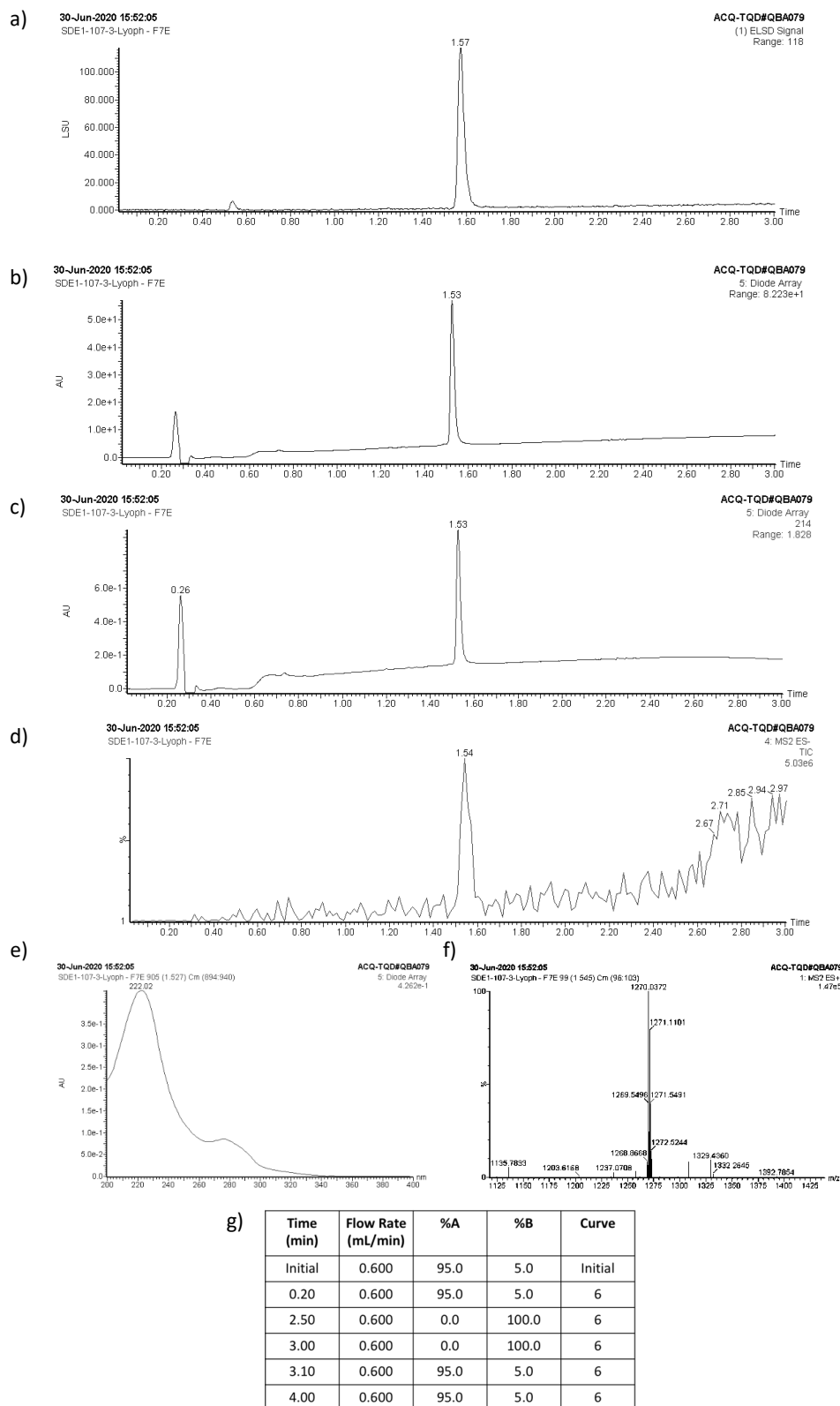Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S17. UPLC analysis of **F9E**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F$_{11E}$**

Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH

Solvent B: Acetonitrile +0,1% HCOOH

Figure S18. UPLC analysis of **F$_{11E}$**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F11SE**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

g)

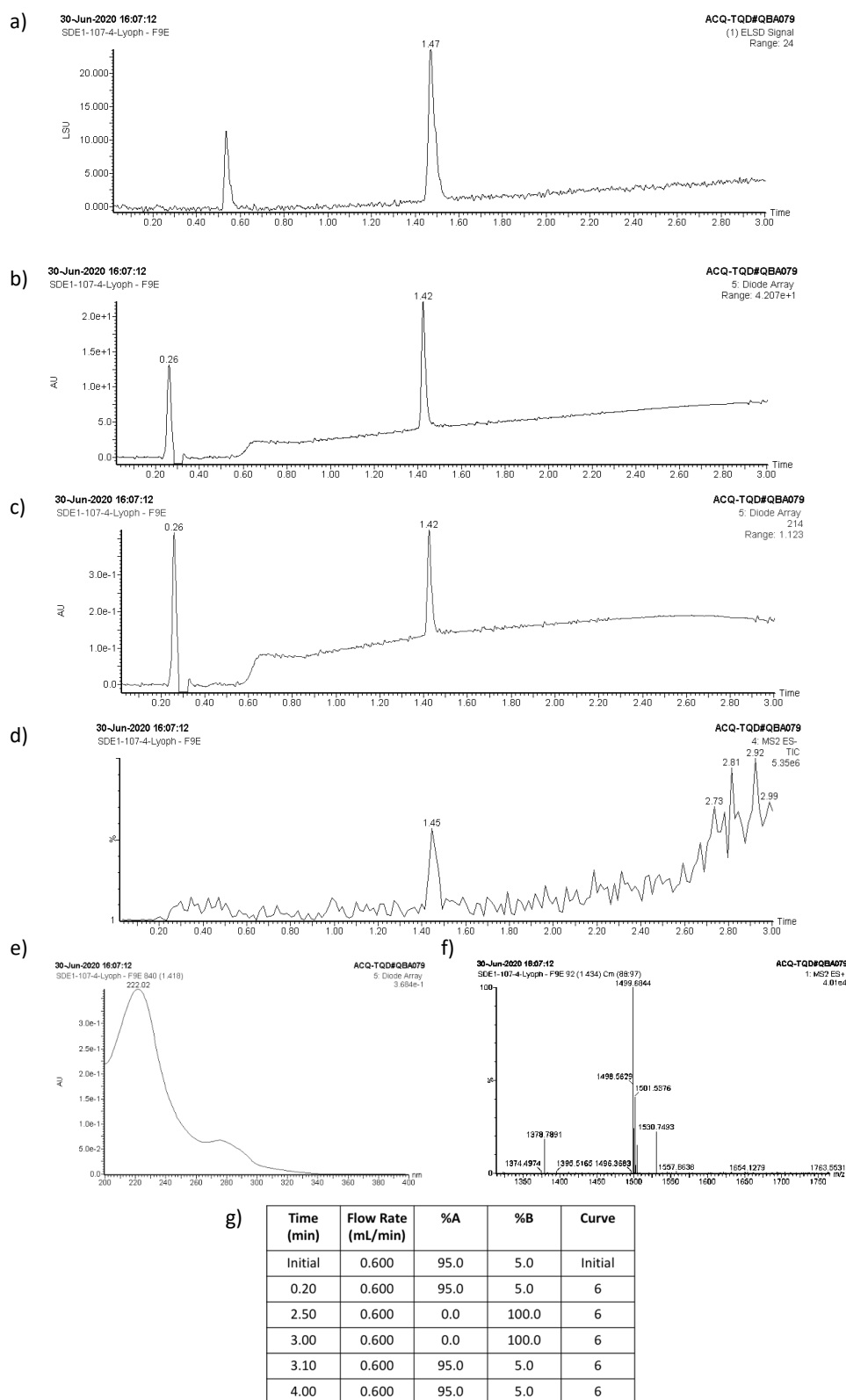| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S19. UPLC analysis of **F11SE**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI$^-$), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **F₁₁ss**

Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH

Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

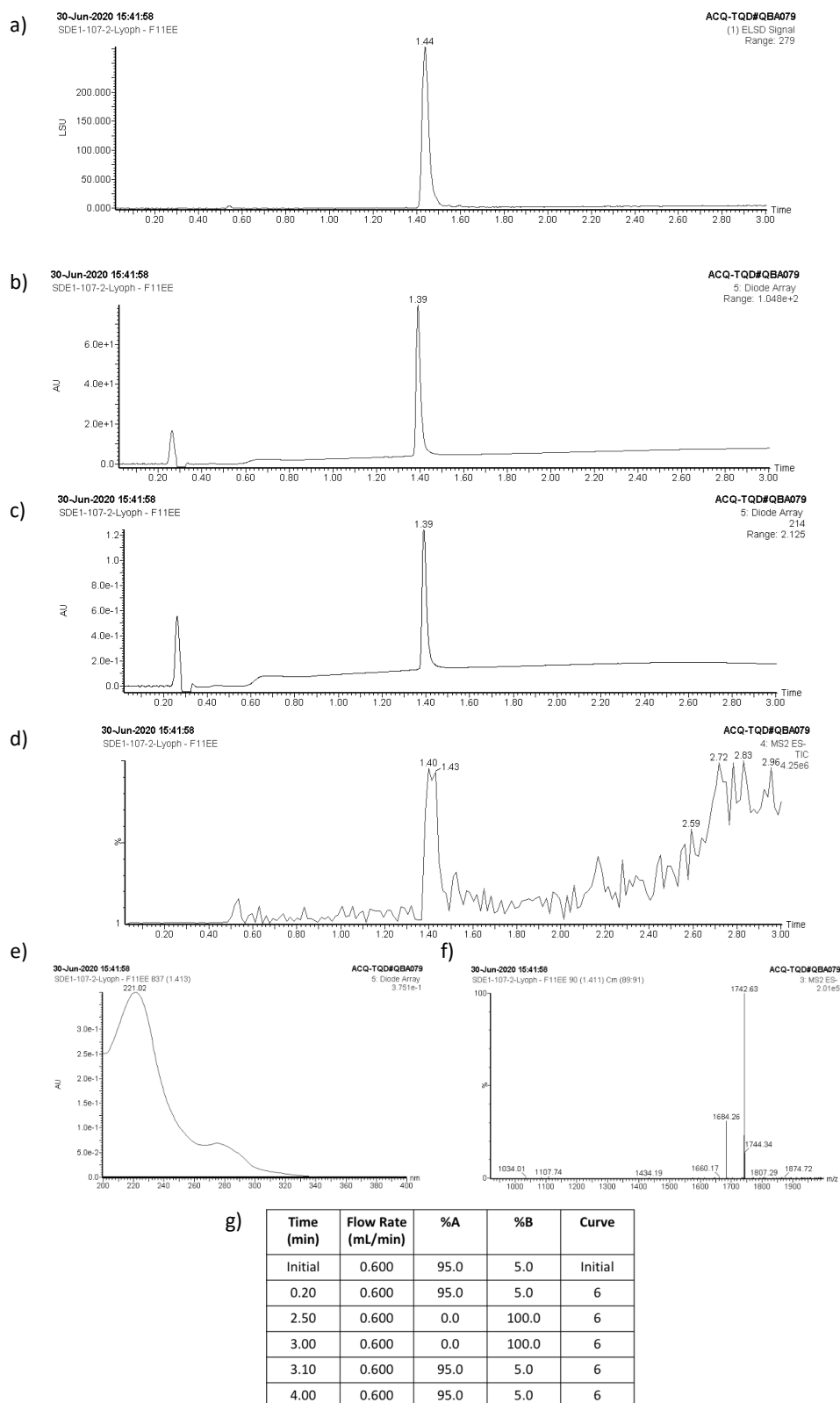| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.10	0.600	95.0	5.0	6
0.20	0.600	80.0	20.0	6
2.50	0.600	70.0	30.0	6
2.55	0.600	0.0	100.0	6
3.0	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S20. UPLC analysis of **F₁₁ss**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **pF₁₁**
Column: BEH C18 2.1x50 mm 1.7 µm

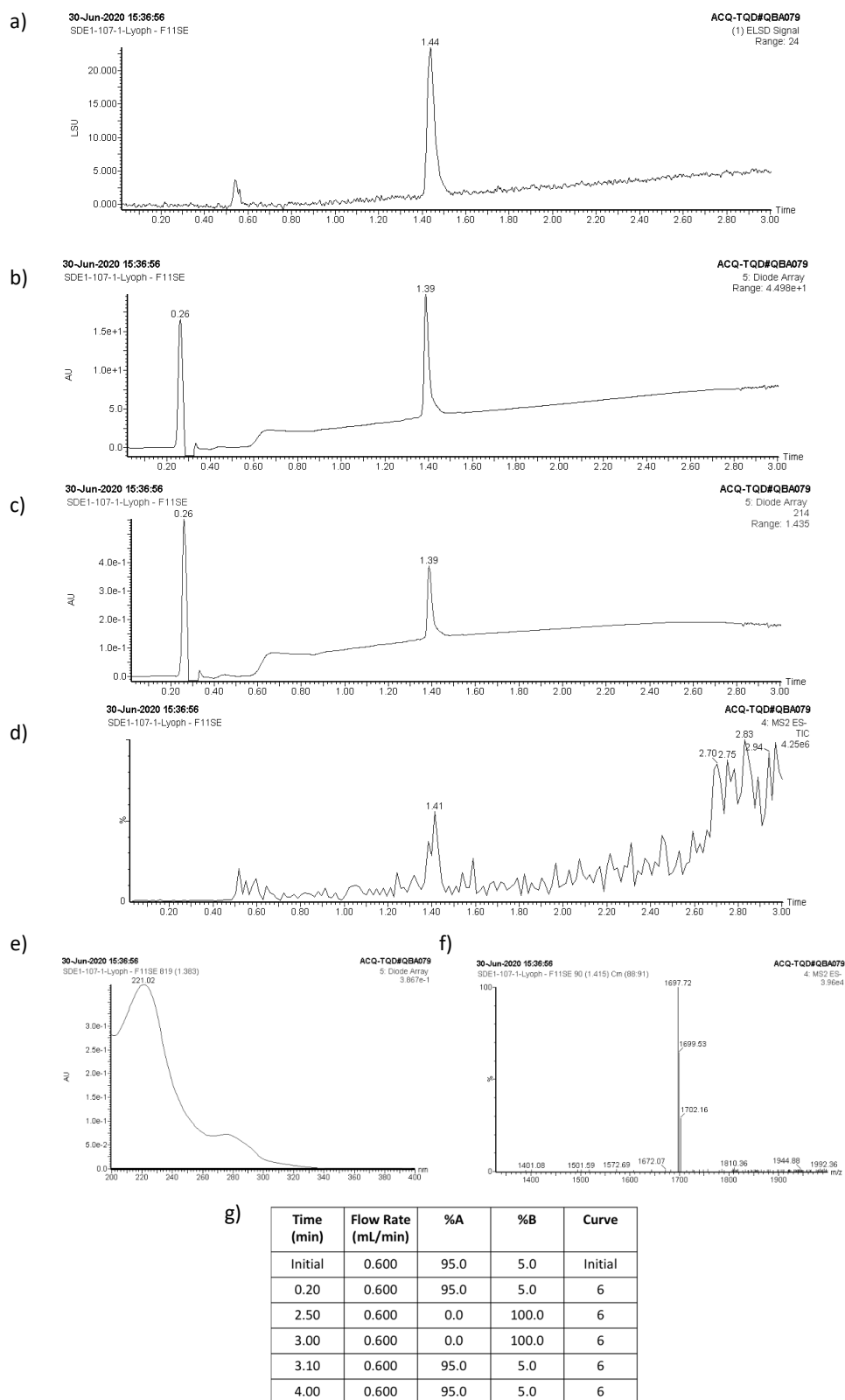Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

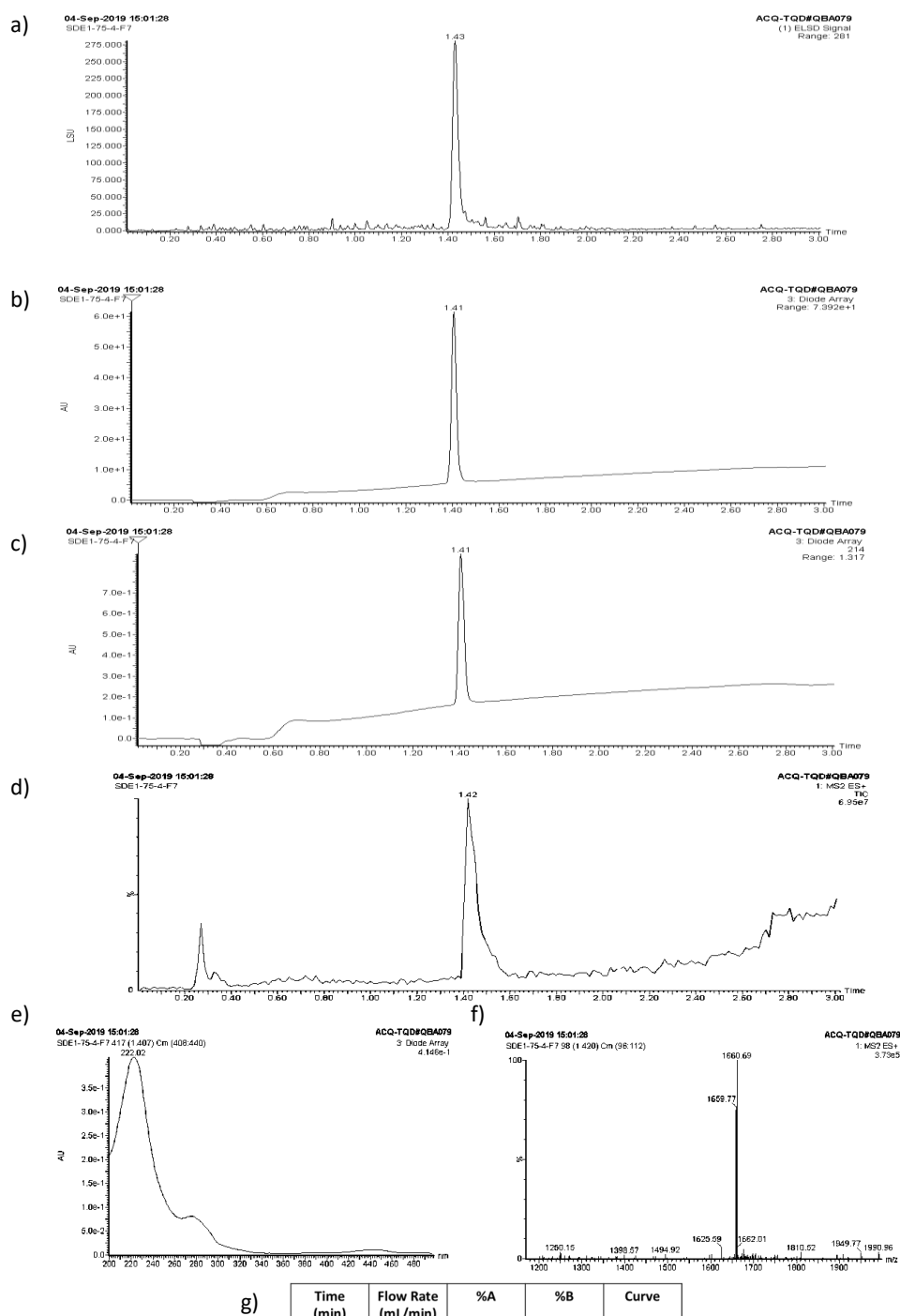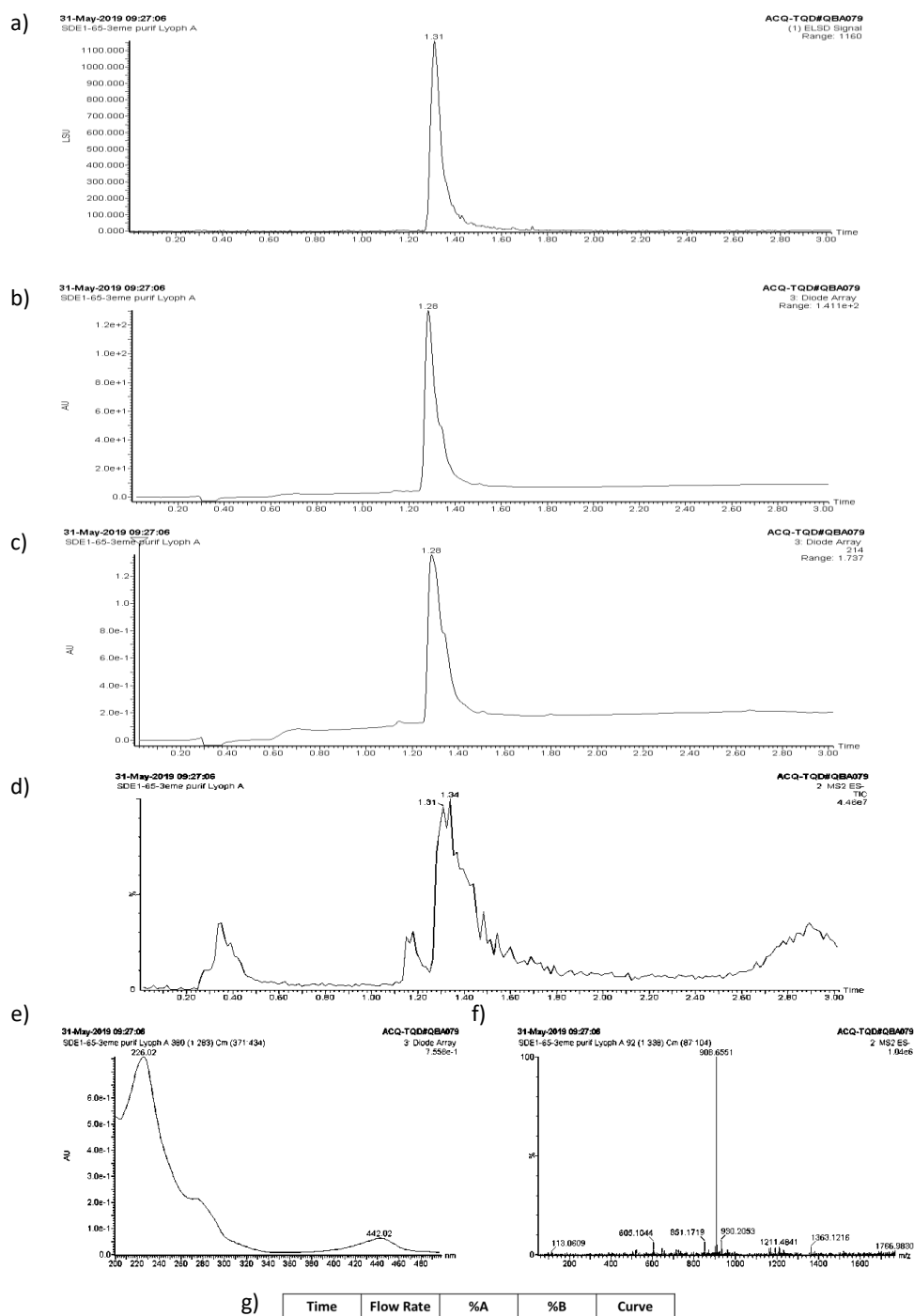|	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
	Initial	0.600	95.0	5.0	Initial
	0.10	0.600	95.0	5.0	6
	0.20	0.600	80.0	20.0	6
	2.50	0.600	70.0	30.0	6
	2.55	0.600	0.0	100.0	6
	3.0	0.600	0.0	100.0	6
	3.10	0.600	95.0	5.0	6
	4.00	0.600	95.0	5.0	6

Figure S21. UPLC analysis of **pF₁₁**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN₅ₑ**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
g)	Initial	0.600	95.0	5.0	Initial
	0.20	0.600	95.0	5.0	6
	2.50	0.600	0.0	100.0	6
	3.00	0.600	0.0	100.0	6
	3.10	0.600	95.0	5.0	6
	4.00	0.600	95.0	5.0	6

Figure S22. UPLC analysis of **BCN₅ₑ**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN$_{6E}$**
Column: BEH C18 2.1x50 mm 1.7 μm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

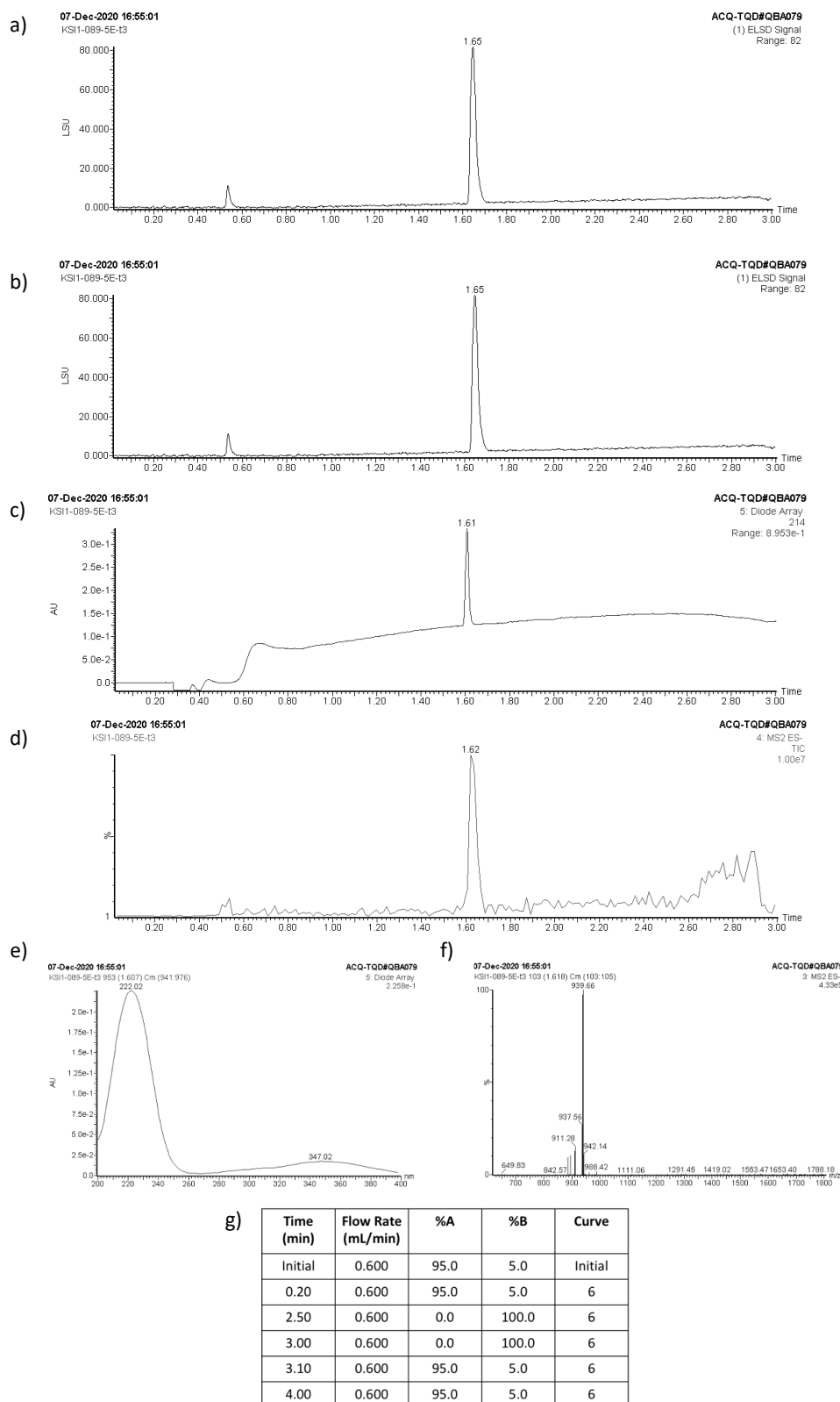| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S23. UPLC analysis of **BCN$_{6E}$**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI$^-$), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN₇ₑ**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

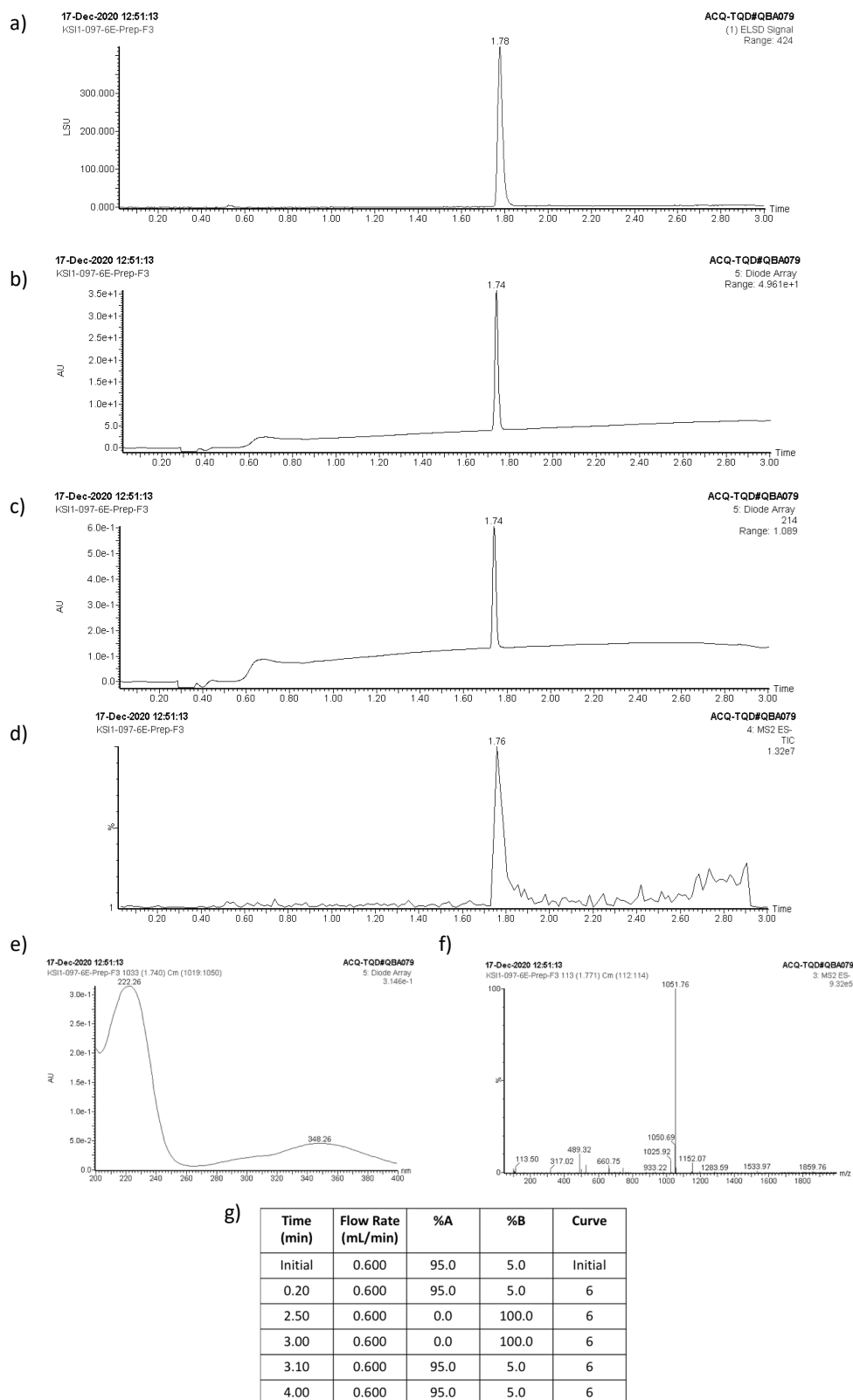| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S24. UPLC analysis of **BCN₇ₑ**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN₈ₑ**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S25. UPLC analysis of **BCN₈ₑ**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN9E**
Column: BEH C18 2.1x50 mm 1.7 µm

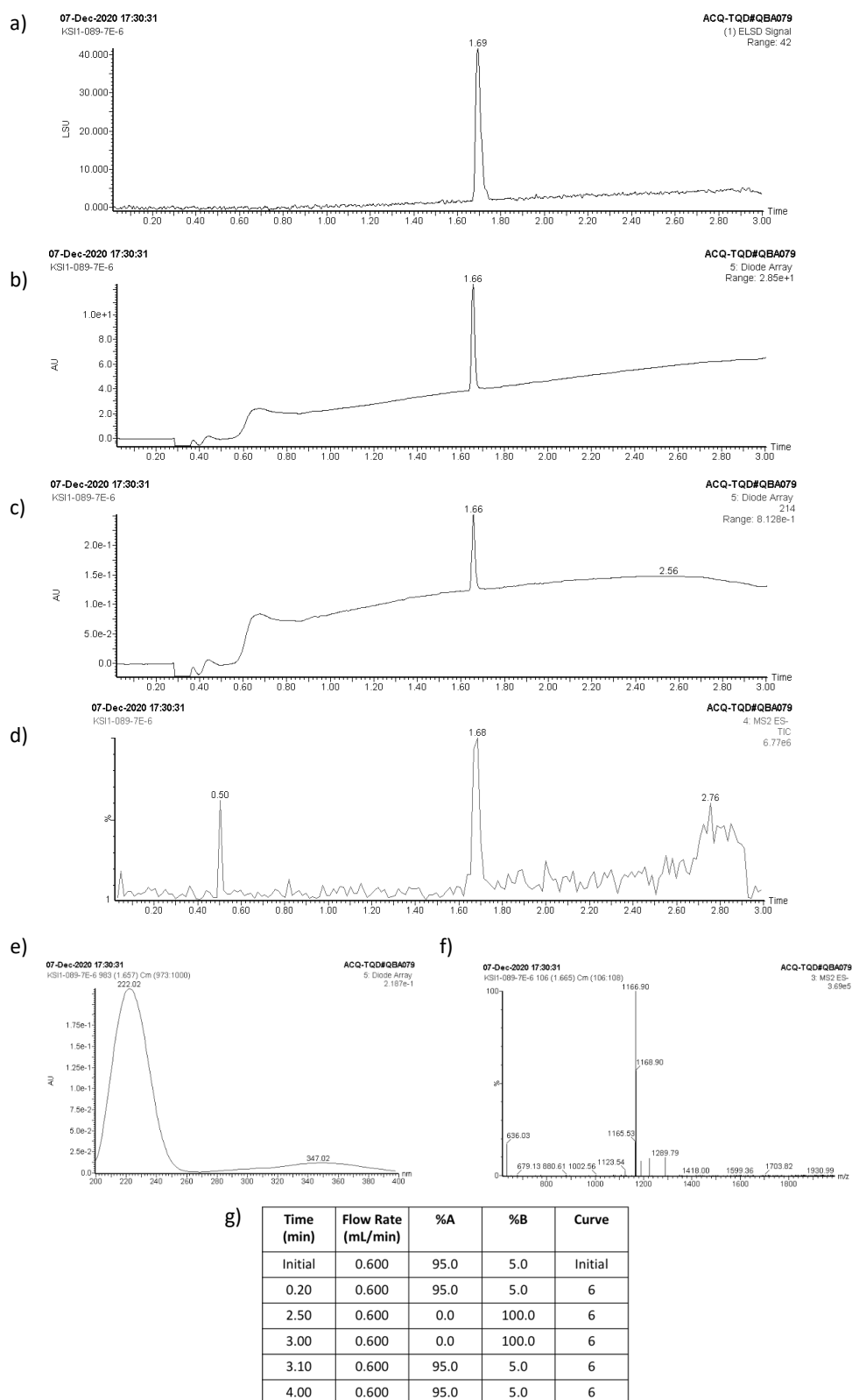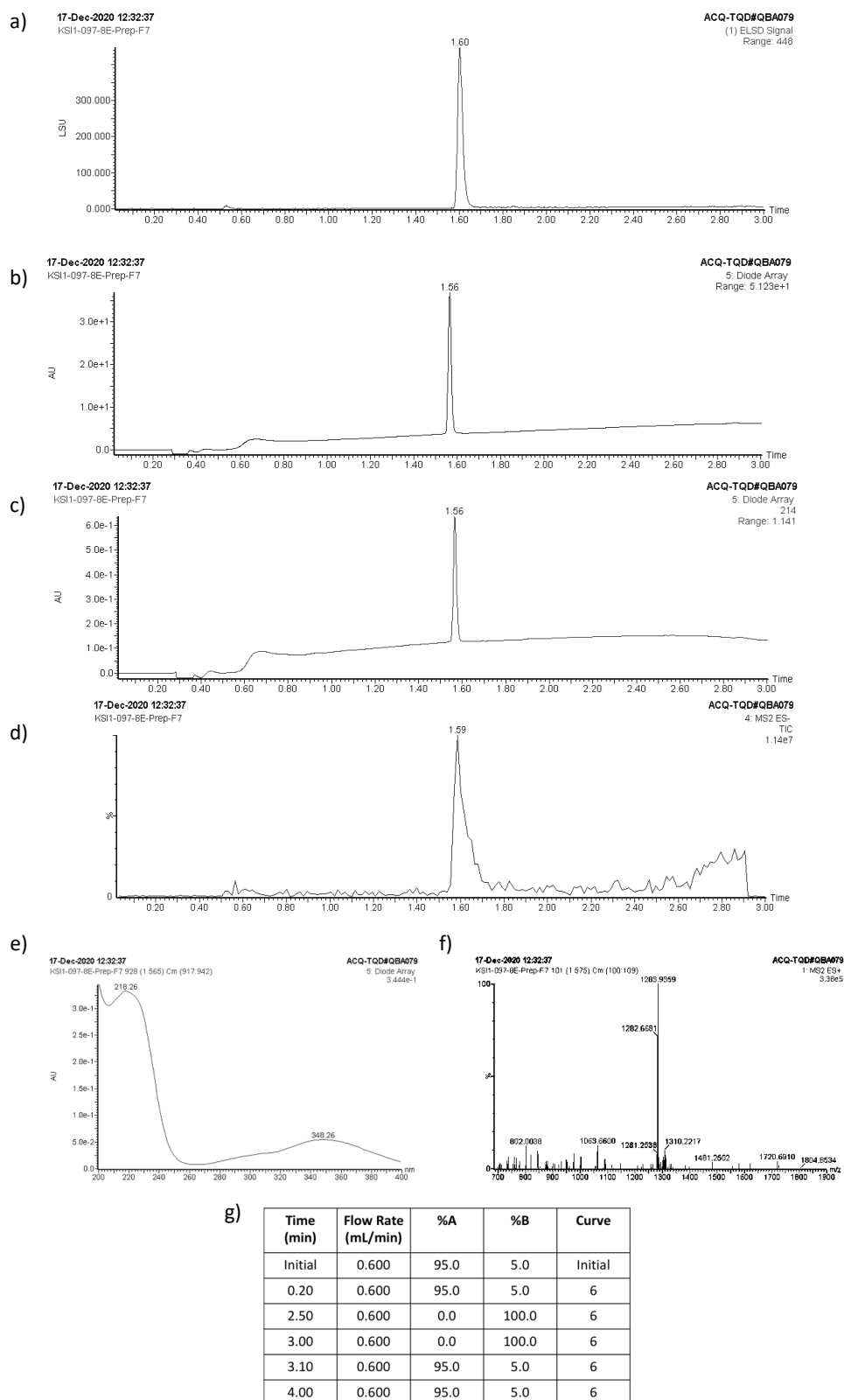Solvent A: H₂O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

17-Dec-2020 13:42:52
KSI1-094-9E-Prep-F4

ACQ-TQD#QBA079
(1) ELSD Signal
Range: 301

1.57

b)

17-Dec-2020 13:42:52
KSI1-094-9E-Prep-F4

ACQ-TQD#QBA079
5: Diode Array
Range: 4.49e+1

1.53

c)

17-Dec-2020 13:42:52
KSI-094-9E-Prep-F4

ACQ-TQD#QBA079
5: Diode Array
214
Range: 1.054

1.53

d)

17-Dec-2020 13:42:52
KSI1-094-9E-Prep-F4

ACQ-TQD#QBA079
2: MS2 ES+
TIC
4.83e7

0.51
1.55
2.88

e)

17-Dec-2020 13:42:52
KSI1-094-9E-Prep-F4 908 (1.532) Cm (856:976)

ACQ-TQD#QBA079
5: Diode Array
1.943e-1

221.26
345.26

f)

17-Dec-2020 13:42:52
KSI1-094-9E-Prep-F4 99 (1.547) Cm (98:103)

ACQ-TQD#QBA079
2: MS2 ES+
2.88e5

1398.15
1397.47
1399.17
1397.27
1397.13
1399.56
1396.93
1404.83
1383.18
1386.98
1395.13
1412.54

g)

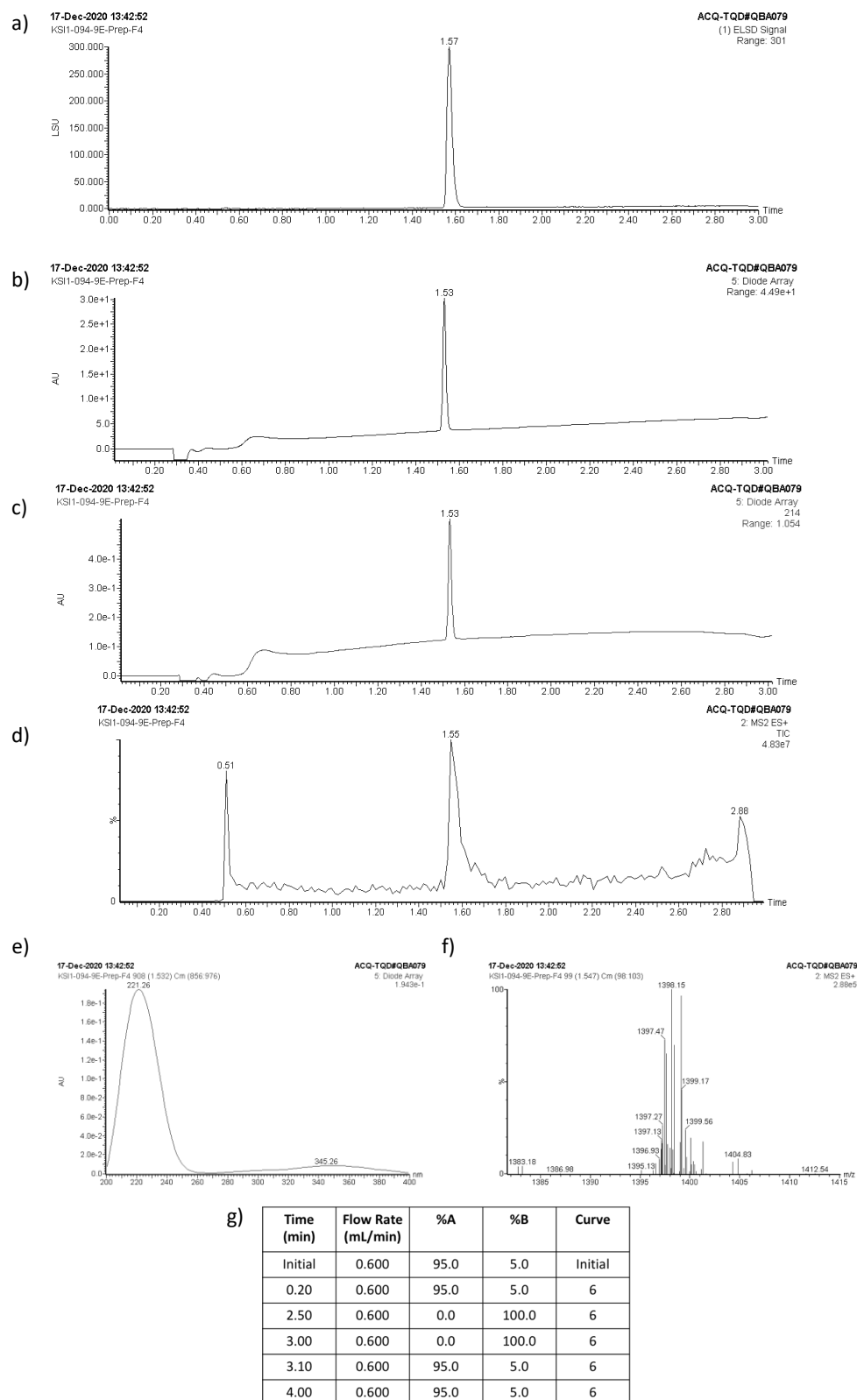| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S26. UPLC analysis of **BCN9E**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁺), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN₁₀EE**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

g)

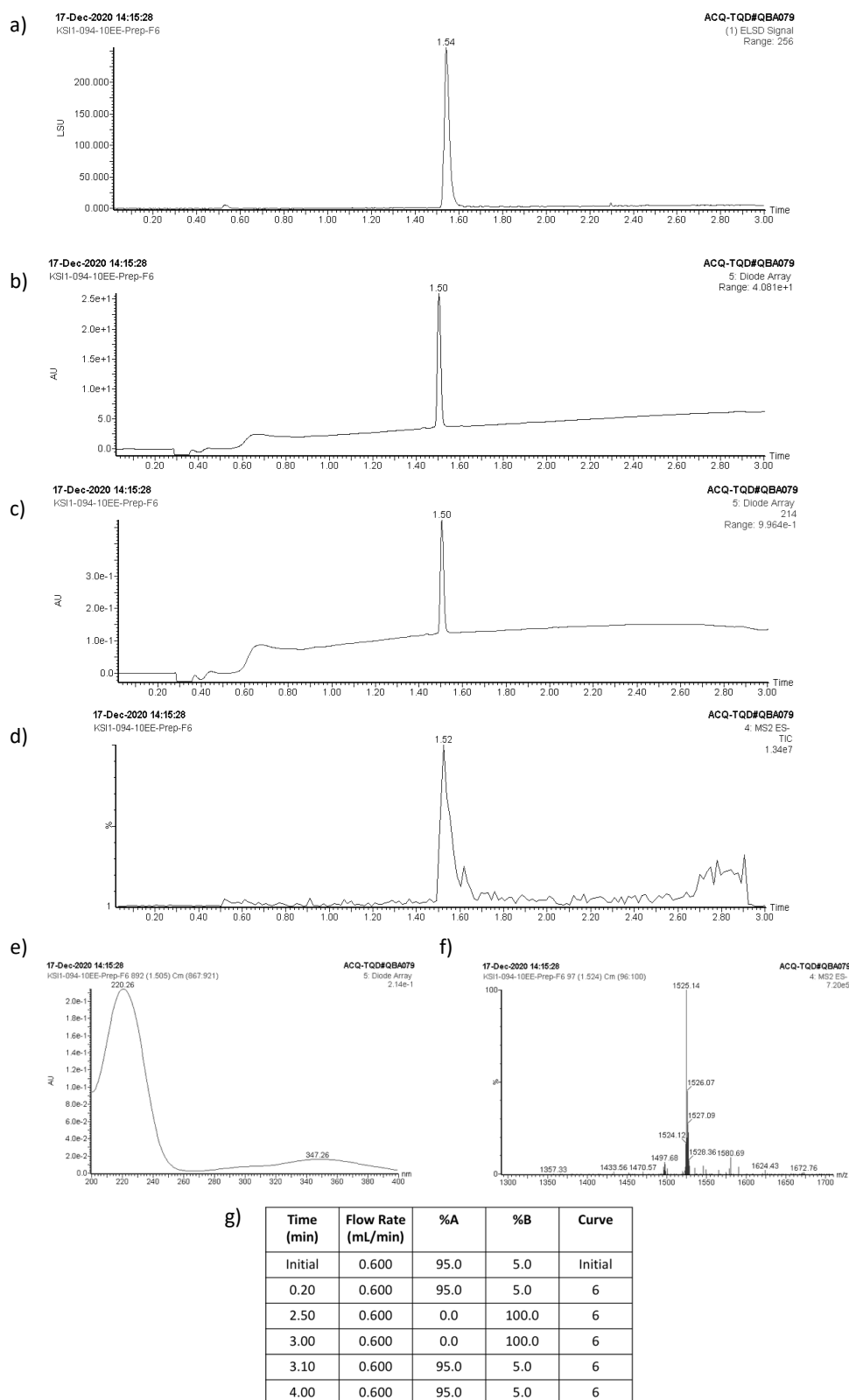| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S27. UPLC analysis of **BCN₁₀EE**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **BCN$_{11EE}$**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

d)

e)

f)

g)

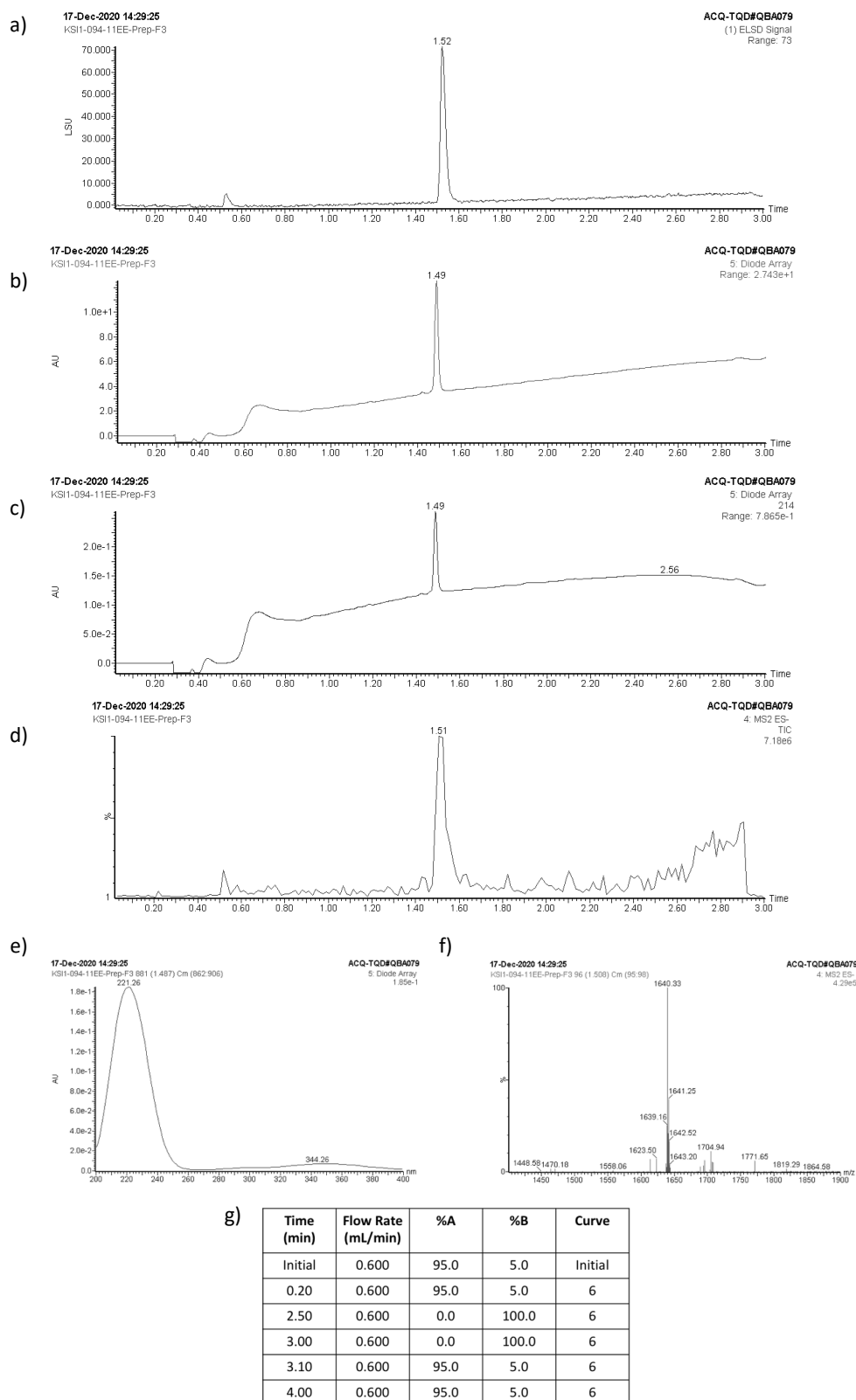| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S28. UPLC analysis of **BCN$_{11EE}$**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI$^-$), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

a)

b)

c)

d)

e)

f)

g)

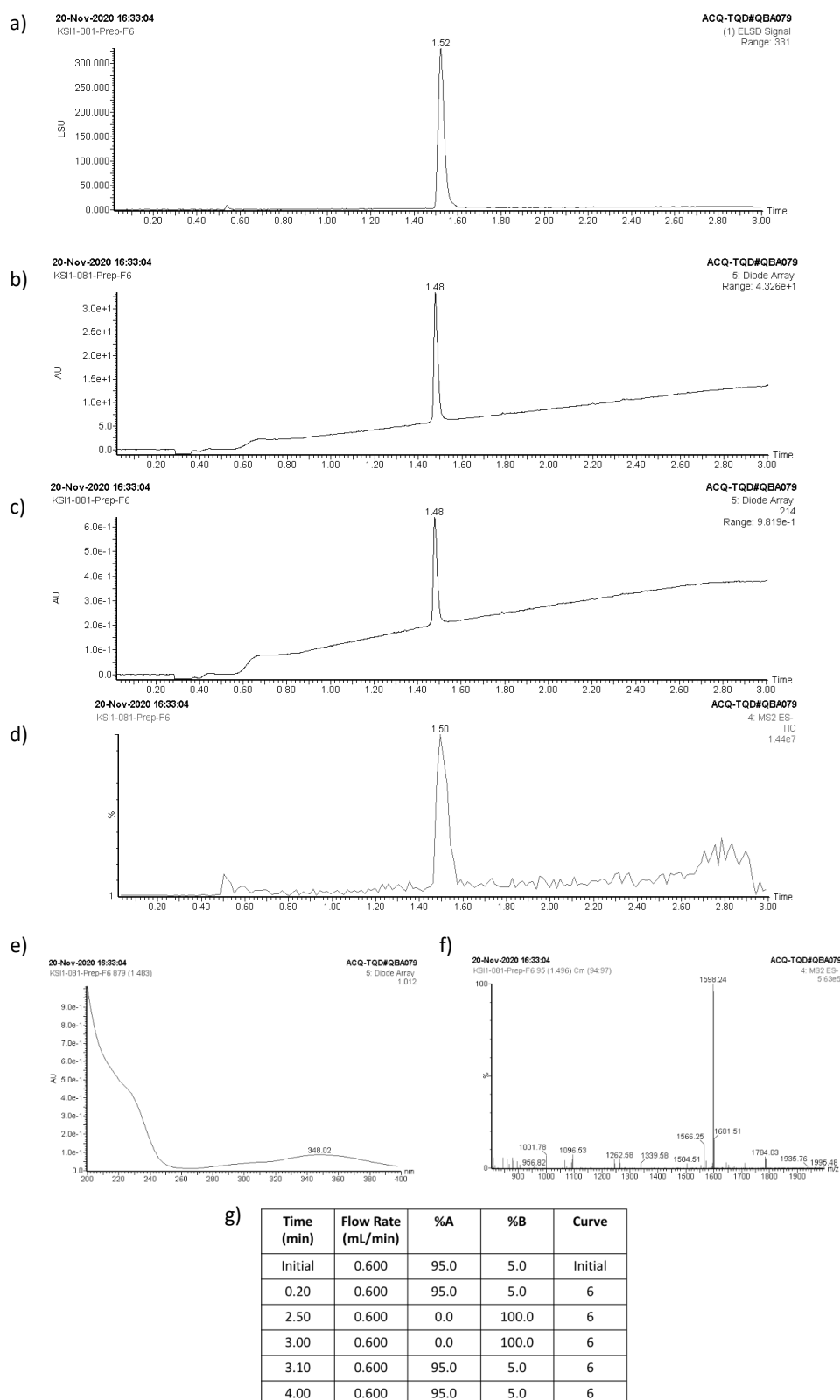| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.20	0.600	95.0	5.0	6
2.50	0.600	0.0	100.0	6
3.00	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S29. UPLC analysis of **BCN₁₁SE**. a) ELSD detector, b) Photodiode array detector (absorbance maximum for each compound between 220–500), c) Photodiode array detector (λ:214nm), d) Mass detector (ESI⁻), e) absorption spectra of the compound, f) Mass spectra of the compound, g) gradient.

Sample name: **Blank**
Column: BEH C18 2.1x50 mm 1.7 µm

Solvent A: H$_2$O+0.1%HCOOH
Solvent B: Acetonitrile +0,1% HCOOH

a)

b)

c)

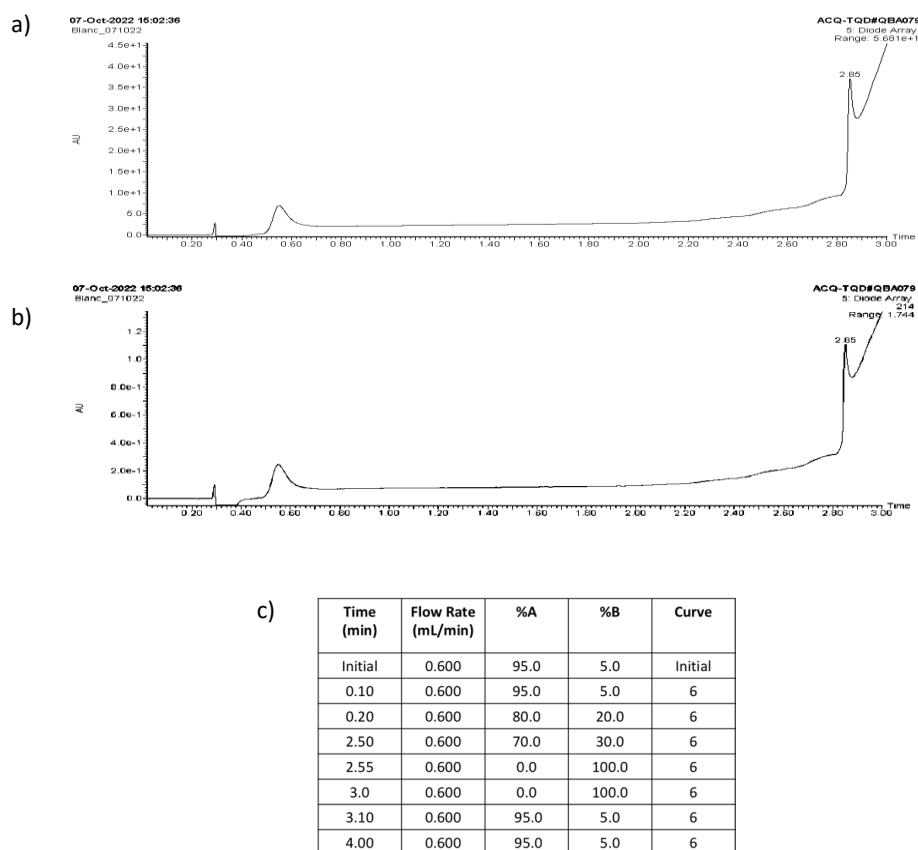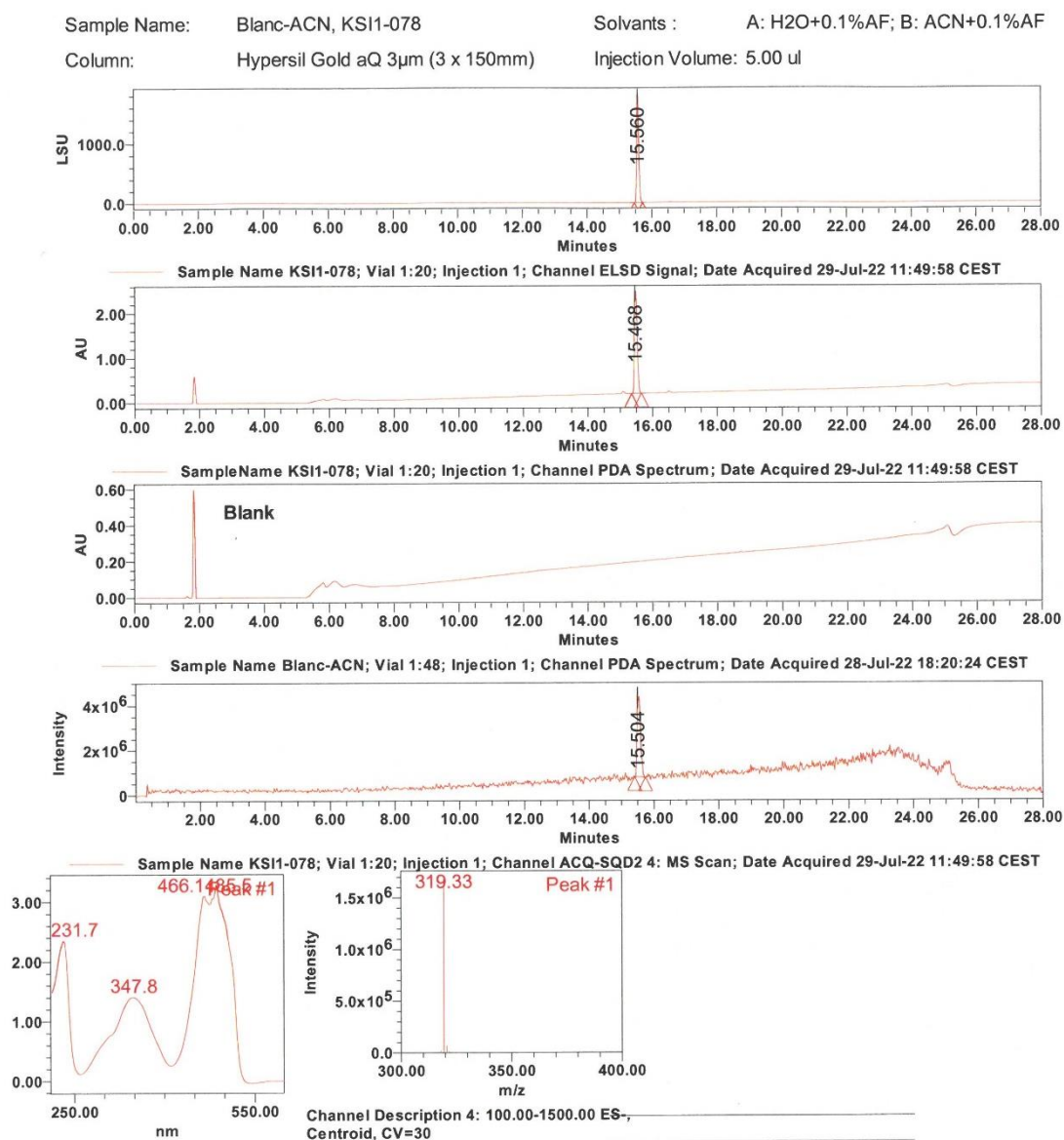| Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.600	95.0	5.0	Initial
0.10	0.600	95.0	5.0	6
0.20	0.600	80.0	20.0	6
2.50	0.600	70.0	30.0	6
2.55	0.600	0.0	100.0	6
3.0	0.600	0.0	100.0	6
3.10	0.600	95.0	5.0	6
4.00	0.600	95.0	5.0	6

Figure S30. UPLC analysis of **Blank**. a) Photodiode array detector (absorbance maximum for each compound between 220–500), b) Photodiode array detector (λ:214nm), c) gradient.
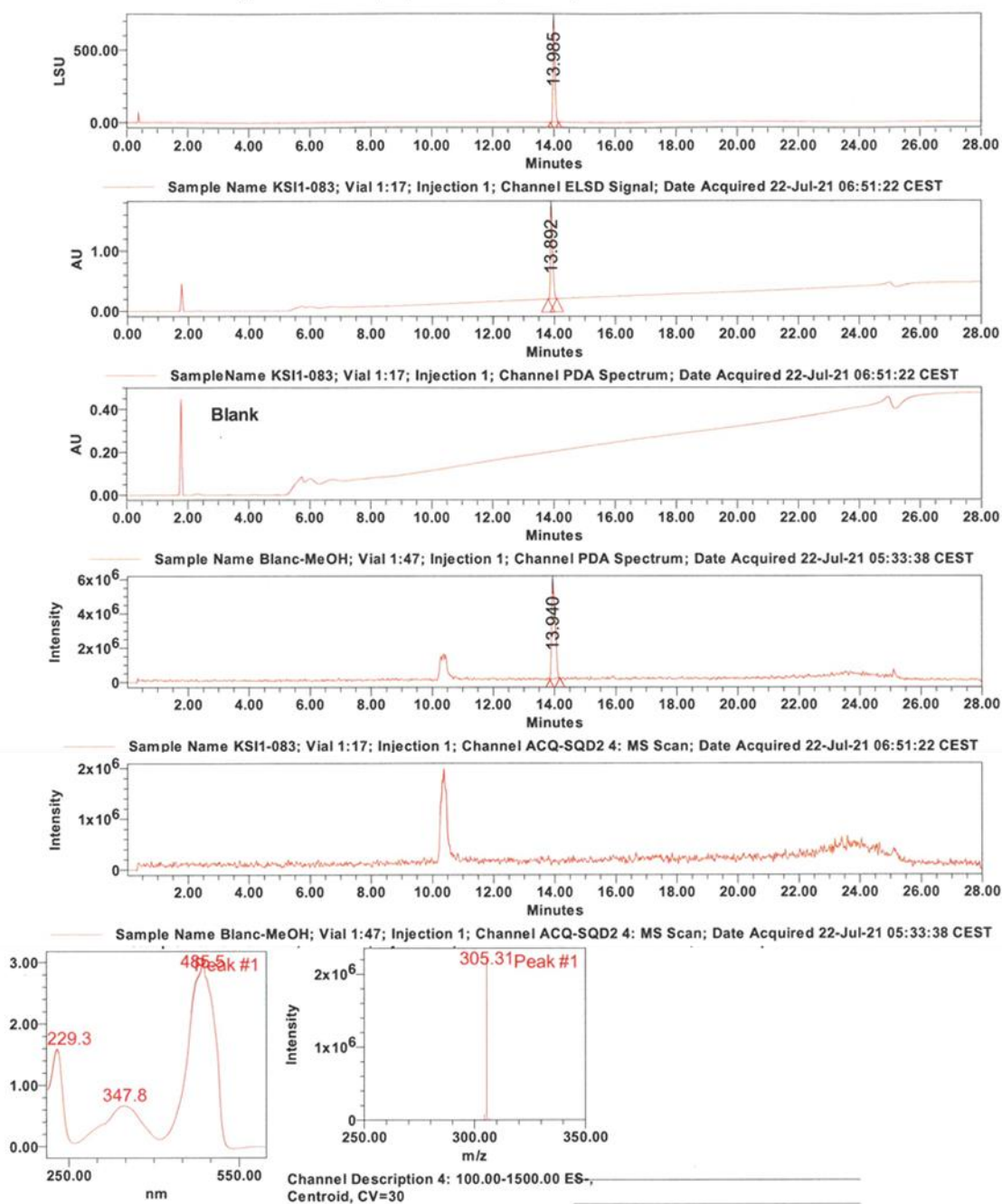
HPLC Traces

Figure S31. HPLC analysis of **7**. from the top to the bottom: ELSD detector, Photodiode Array Detector, Blank with Photodiode Array Detector and Mass detector (ESI⁻), absorption spectra of the compound (left), Mass spectra of the compound (right) and gradient.

Sample Name: KSI1-083, Blanc-MeOH   Solvents :   A: H2O+0.1%AF; B: ACN+0.1%AF

Column: Hypersil Gold aQ 3µm (3 x 150mm)   Injection Volume: 5.00 ul

Sample Name KSI1-083; Vial 1:17; Injection 1; Channel ELSD Signal; Date Acquired 22-Jul-21 06:51:22 CEST

SampleName KSI1-083; Vial 1:17; Injection 1; Channel PDA Spectrum; Date Acquired 22-Jul-21 06:51:22 CEST

Blank

Sample Name Blanc-MeOH; Vial 1:47; Injection 1; Channel PDA Spectrum; Date Acquired 22-Jul-21 05:33:38 CEST

Sample Name KSI1-083; Vial 1:17; Injection 1; Channel ACQ-SQD2 4: MS Scan; Date Acquired 22-Jul-21 06:51:22 CEST

Sample Name Blanc-MeOH; Vial 1:47; Injection 1; Channel ACQ-SQD2 4: MS Scan; Date Acquired 22-Jul-21 05:33:38 CEST

Channel Description 4: 100.00-1500.00 ES-, Centroid, CV=30

	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.500	100.0	0.0	0.0	0.0	Initial
2	3.00	0.500	100.0	0.0	0.0	0.0	6
3	23.00	0.500	0.0	100.0	0.0	0.0	6
4	28.00	0.500	0.0	100.0	0.0	0.0	6
5	29.00	0.500	100.0	0.0	0.0	0.0	6
6	39.00	0.500	100.0	0.0	0.0	0.0	6
7	40.00	0.000	100.0	0.0	0.0	0.0	6

Figure S32. HPLC analysis of **5**. from the top to the bottom: ELSD detector, Photodiode Array Detector, Blank with Photodiode Array Detector, Mass detector (ESI⁻), Blank with Mass detector (ESI⁻), absorption spectra of the compound (left), Mass spectra of the compound (right) and gradient.
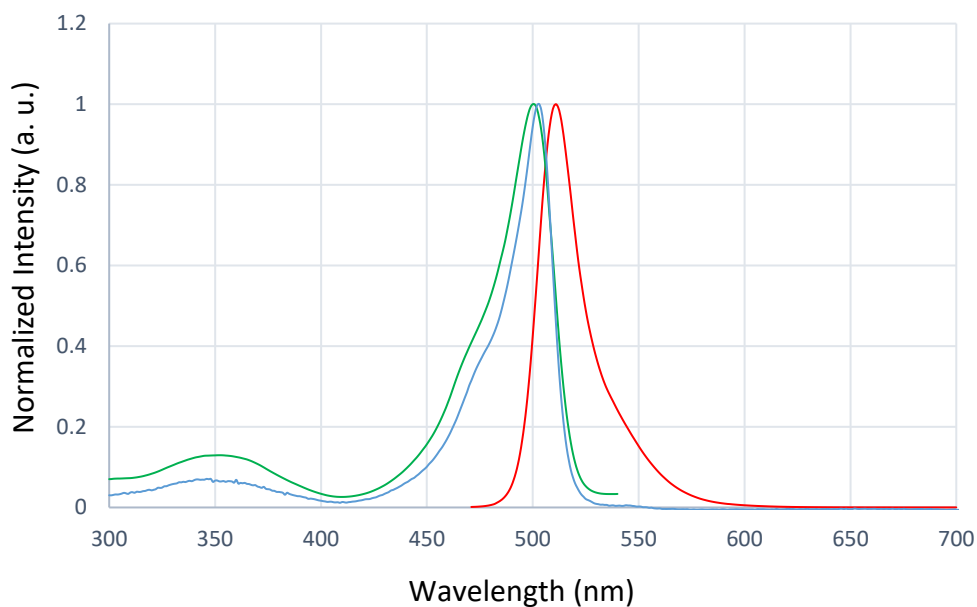
UV-Visible Absorption and fluorescence Spectra

Figure S33. UV-Visible absorption Spectrum (blue) of **BCN$_{10EE}$** [5.00 μM] in Tris buffer 20mM pH:7.8, recorded on Varian Cary 100 spectrophotometer. Fluorescence excitation (green, λ_{em}= 350nm) and emission spectra (red, λ_{ex} = 450nm) of **BCN$_{10EE}$** [2.50 μM] in Tris buffer 20mM pH:7.8 at 25°C recorded on HITACI F-2500 spectrofluorometer.