Supplemental Information

Stationary Phase Effects in Hydrophilic Interaction Liquid Chromatographic Separation of Oligonucleotides

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Supplemental Information Provided:

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Supplemental Table S1. Molecular weight (MW) and electrospray series values for $tRNA^{Phe}$ from yeast for the digestion products of just RNase T1 and RNase T1 with bacterial alkaline phosphatase (BAP). Values only reported that align with the scan range employed for mass spectrometry (600Da-2000Da).

RNase T1 Dig. Products	MW	-1	-2	-3	-4	-5
CGp	668.4	667.4				
UGp	669.4	668.4				
AGp	692.4	691.4				
DDGp	979.6	978.6				
[m^5U]ΨCGp	1294.8	1293.8	646.4			
CCAGp	1302.8	1301.8	650.4			
CACCA	1512.0	1511.0	755.0			
CUCAGp	1609.0	1608.0	803.5			
AAUUCGp	1939.2	1938.2	968.6	645.4		
AUUUA[m^2G]p	1954.2	1953.2	976.1	650.4		
[m^7G]UC[m^5C]UGp	1959.2	1958.2	978.6	652.1		
[m^1A]UCCACAGp	2586.6		1292.3	861.2	645.6	
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UGp	4166.7			1387.9	1040.7	832.3

RNase T1 + BAP Dig. Products	MW	-1	-2	-3	-4	-5
CG	588.4	587.4				
UG	589.4	588.4				
AG	612.5	611.4				
DDG	899.6	898.6				
[m^5U]ΨCG	1214.8	1213.8	606.4			
CCAG	1222.8	1221.8	610.4			
CACCA	1512.0	1511.0	755.0			
CUCAG	1529.0	1528.0	763.5			
AAUUCG	1859.2	1858.2	928.6	618.7		
AUUUA[m^2G]	1874.2	1873.2	936.1	623.7		
[m^7G]UC[m^5C]UG	1879.2	1878.2	938.6	625.4		
[m^1A]UCCACAG	2506.6		1252.3	834.5	625.6	
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UG	4086.7			1361.2	1020.7	816.3

Supplemental Table S2. Chromatographic Figures of Merit for the comparison of the five HILIC columns that perform best for the LC-MS analysis of $tRNA^{Phe}$ RNase T1 digestion products. 4 μg of digest was injected. Digestion products were identified through extracted ion chromatograms (EICs) of the most abundant charge state and each was confirmed by MS/MS sequence data. If no figures of merit are listed, then the RNase T1 digestion product was not detected in the LC-MS/MS data. Figures of merit reported are retention factor (k'), resolution (R), Peak Capacity (P_c), peak symmetry (S), and peak width at half height ($w_{1/2}$).

Waters BEH Amide

Digestion Product	Retention Factor (k')	Resolution (R)	Peak Capacity (P_c)	Symmetry (S)	$w_{1/2}$ (min)
CGp	4.2	0.2	104	2.0	0.2
UGp	4.2	0.0	104	2.0	0.2
AGp	4.1	---	53	2.0	0.4
DDGp	7.0	6.7	53	0.7	0.4
[m^5U]ΨCGp	10.6	4.0	53	1.7	0.4
CCAGp	10.7	0.1	53	1.7	0.4
CACCA	8.3	2.4	53	1.0	0.4
CUCAGp	12.3	1.0	70	2.0	0.3
AAUUCGp	13.1	1.5	53	2.5	0.4
AUUUA[m^2G]p	11.9	2.5	70	2.0	0.3
[m^7G]UC[m^5C]UGp	14.7	2.8	53	1.3	0.4
[m^1A]UCCACAGp	15.7	1.9	60	2.0	0.35
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UGp	15.7	0.0	60	2.0	0.35

Shodex VN-50

Digestion Product	Retention Factor (k')	Resolution (R)	Peak Capacity (P_c)	Symmetry (S)	$W_{1/2}$ (min)
CGp	3.0	0.4	53	1.6	0.4
UGp	2.3	---	53	1.3	0.4
AGp	2.7	0.6	35	1.6	0.6
DDGp	3.1	0.1	35	1.6	0.6
[m^5U]ΨCGp	6.0	3.4	35	1.4	0.6
CCAGp	7.6	1.7	35	1.6	0.6
CACCA	6.2	0.2	35	1.4	0.6
CUCAGp	9.1	2.6	104	2.0	0.2
AAUUCGp	10.4	0.3	35	1.6	0.6
AUUUA[m^2G]p	9.1	0.2	104	2.0	0.2
[m^7G]UC[m^5C]UGp	10.1	1.9	35	1.6	0.6
[m^1A]UCCACAGp	12.8	3.7	70	2.0	0.3
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UGp	13.7	2.3	70	1.3	0.3

Waters Torus Diol

Digestion Product	Retention Factor (k')	Resolution (R)	Peak Capacity (P_c)	Symmetry (S)	$w_{1/2}$ (min)
CGp	3.1	0.3	42	1.5	0.5
UGp	2.6	---	42	1.0	0.5
AGp	2.9	0.4	35	3.0	0.6
DDGp	3.8	0.9	42	1.0	0.5
[m^5U]ΨCGp	5.6	0.2	35	2.0	0.6
CCAGp	7.2	1.8	35	1.8	0.6
CACCA	5.4	2.2	35	2.0	0.6
CUCAGp	8.6	1.5	30	2.0	0.7
AAUUCGp	10.0	1.3	30	1.6	0.7
AUUUA[m^2G]p	8.7	0.1	30	2.0	0.7
[m^7G]UC[m^5C]UGp	10.0	0.0	30	1.6	0.7
[m^1A]UCCACAGp	13.0	3.5	42	2.0	0.5
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UGp	14.5	2.0	35	2.3	0.6

Hilicon iHILIC-Fusion

Digestion Product	Retention Factor (k')	Resolution (R)	Peak Capacity (P_c)	Symmetry (S)	$w_{1/2}$ (min)
CGp	2.1	---	104	1.0	0.2
UGp	2.1	0.0	104	1.0	0.2
AGp	2.3	0.6	70	1.0	0.3
DDGp	2.5	0.4	53	1.0	0.4
[m^5U]ΨCGp	4.6	3.4	53	1.3	0.4
CCAGp	5.2	0.7	35	1.3	0.6
CACCA	3.2	1.6	104	1.0	0.2
CUCAGp	7.5	1.7	70	1.7	0.3
AAUUCGp	8.0	1.2	70	1.3	0.3
AUUUA[m^2G]p	6.5	1.8	42	1.4	0.5
[m^7G]UC[m^5C]UGp	9.6	3.9	70	1.3	0.3
[m^1A]UCCACAGp	11.4	5.1	104	1.3	0.2
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UGp	11.5	0.2	104	1.3	

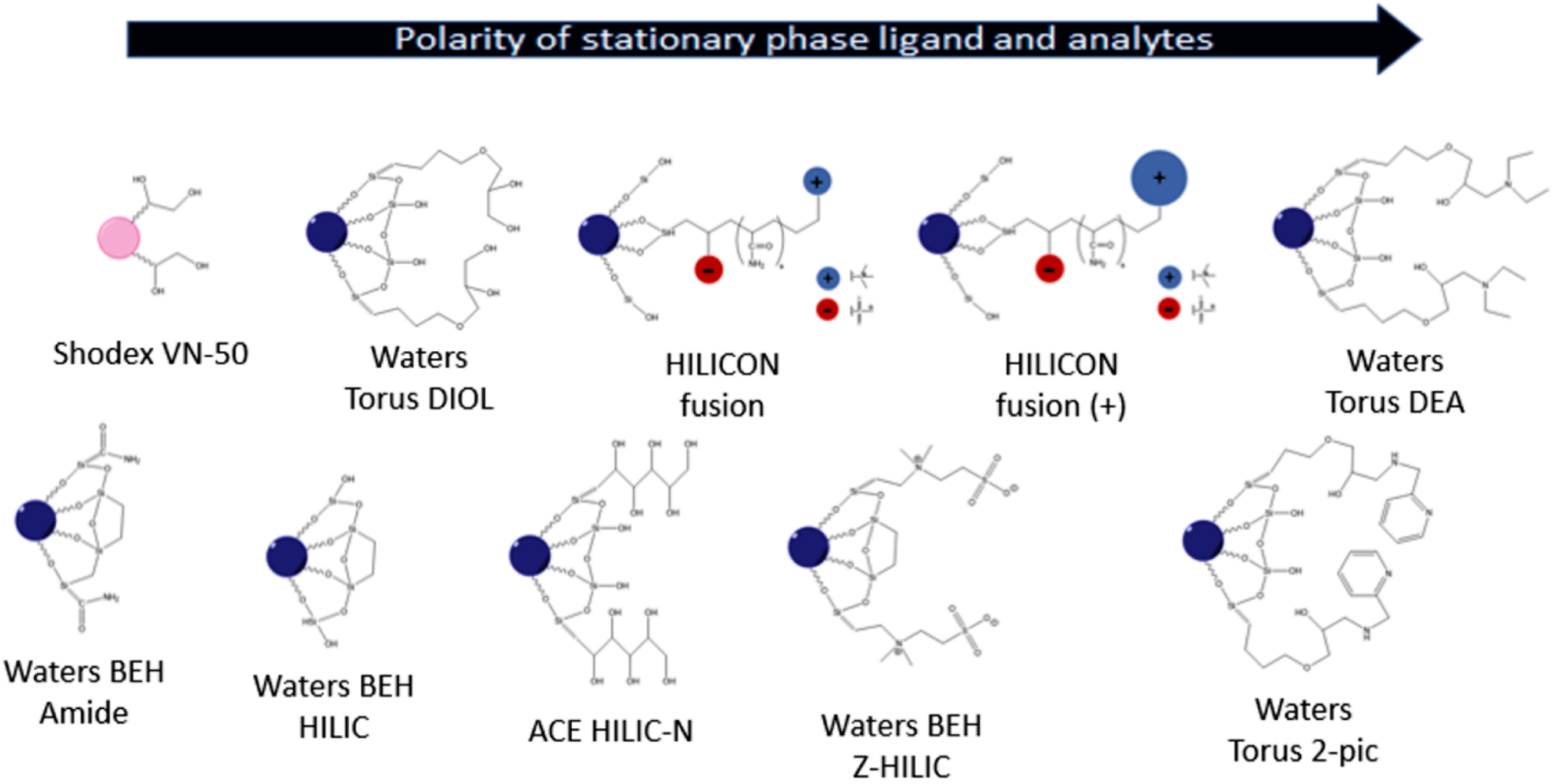
Waters BEH Z-HILIC

Digestion Product	Retention Factor (k')	Resolution (R)	Peak Capacity (P_c)	Symmetry (S)	$w_{1/2}$ (min)
CGp	10.1	0.0	35	1.5	0.6
UGp	10.1	1.2	35	1.5	0.6
AGp	9.1	---	35	0.7	0.6
DDGp	13.4	4.7	53	2.0	0.4
[m^5U]ΨCGp	17.9	6.7	42	1.0	0.5
CCAGp	19.3	1.9	42	1.0	0.5
CACCA	14.2	1.5	70	0.5	0.3
CUCAGp	22.9	0.1	22	1.3	1
AAUUCGp	25.6	0.9	22	1.3	1
AUUUA[m^2G]p	22.8	3.8	27	1.3	0.8
[m^7G]UC[m^5C]UGp	24.3	1.0	22	1.3	1
[m^1A]UCCACAGp	31.9	3.0	11	1.3	2
A[Cm]U[Gm]AA[yW]AΨ[m^5C]UGp	ND	ND	ND	ND	ND

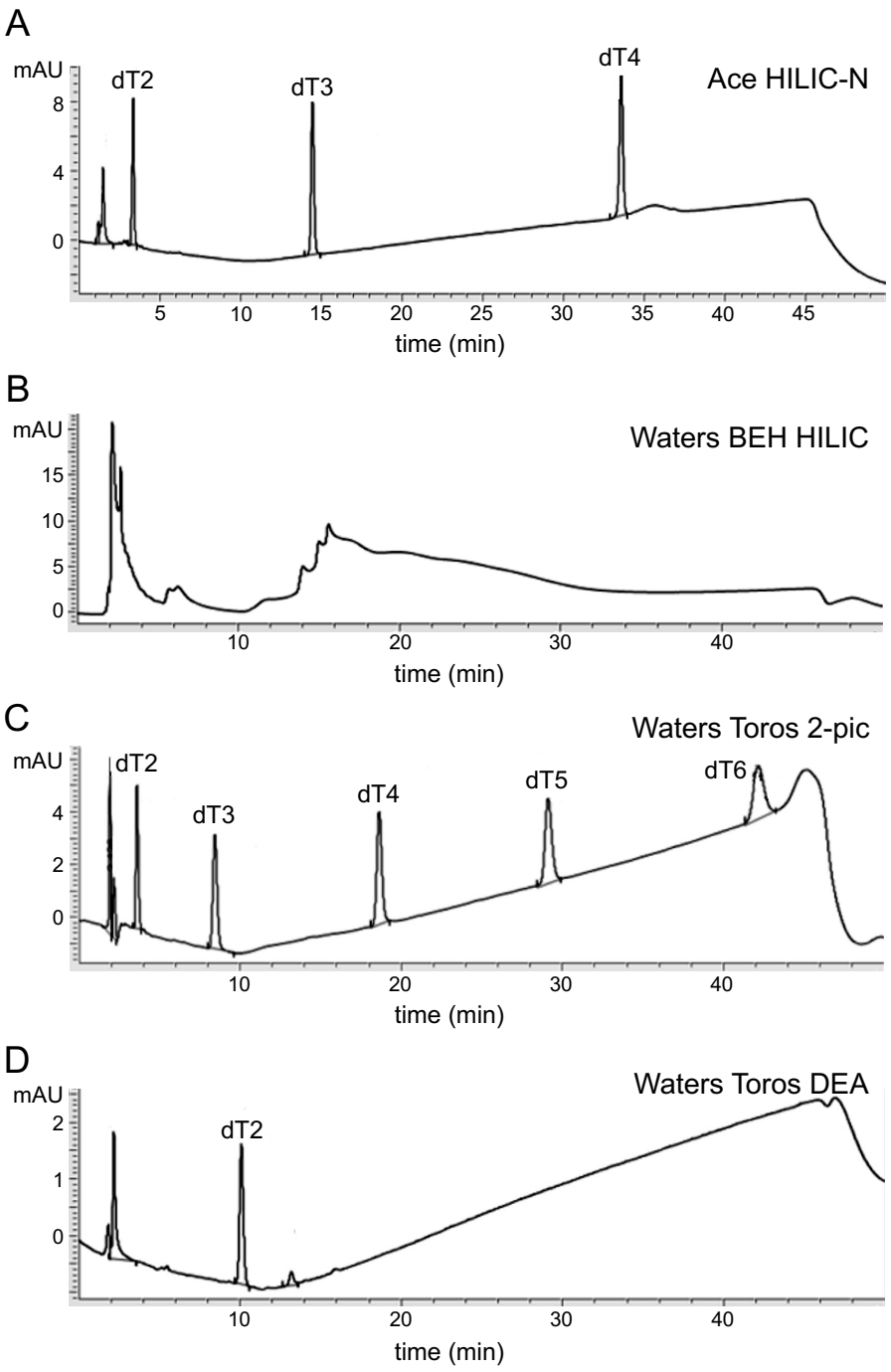
Supplemental Table S3. Retention factors for RNase T1 digestion products of *S. cerevisiae* tRNA-Phe after treatment with bacterial alkaline phosphatase. 4 μg of digest was injected. Digestion products were identified through extracted ion chromatograms (EICs) of the most abundant charge state and each was confirmed by MS/MS sequence data. If no retention factor is listed, then the RNase T1 digestion product was not detected in the LC-MS/MS data.

Yeast tRNA^{Phe} RNase T1 + BAP Digestion products	Waters BEH amide	Shodex VN-50	Waters Torus Diol	HILICON iHILIC-Fusion	Waters BEH Z-HILIC	HILICON iHILIC-Fusion+	Waters Torus 2-PIC
AG	3.1	---	3.0	2.3	4.3	2.6	4.2
DDG	3.8	---	3.2	2.5	8.9	3.6	6.9
[m^{5}U]ΨCG	7.0	4.5	4.6	3.4	15.2	8.4	16.9
CCAG	7.3	5.7	5.0	3.6	16.7	10.7	18.3
CACCA	8.8	7.9	7.2	4.8	17.9	14.9	27.8
CUCAG	10.0	9.0	6.4	4.4	19.3	14.1	27.9
AAUUCG	11.9	10.7	9.0	7.5	21.6	19.9	---
AUUUA[m^{2}G]	8.9	10.5	7.1	4.3	19.0	19.7	---
[m^{7}G]UC[m^{5}C]UG	14.4	9.8	9.1	9.1	22.4	19.9	---
[m^{1}A]UCCACAG	16.1	14.8	14.3	12.4	28.4	---	---
A[Cm]U[Gm]AA[yW]AΨ[m^{5}C]UG	16.1	16.1	16.5	13.3	---	---	---

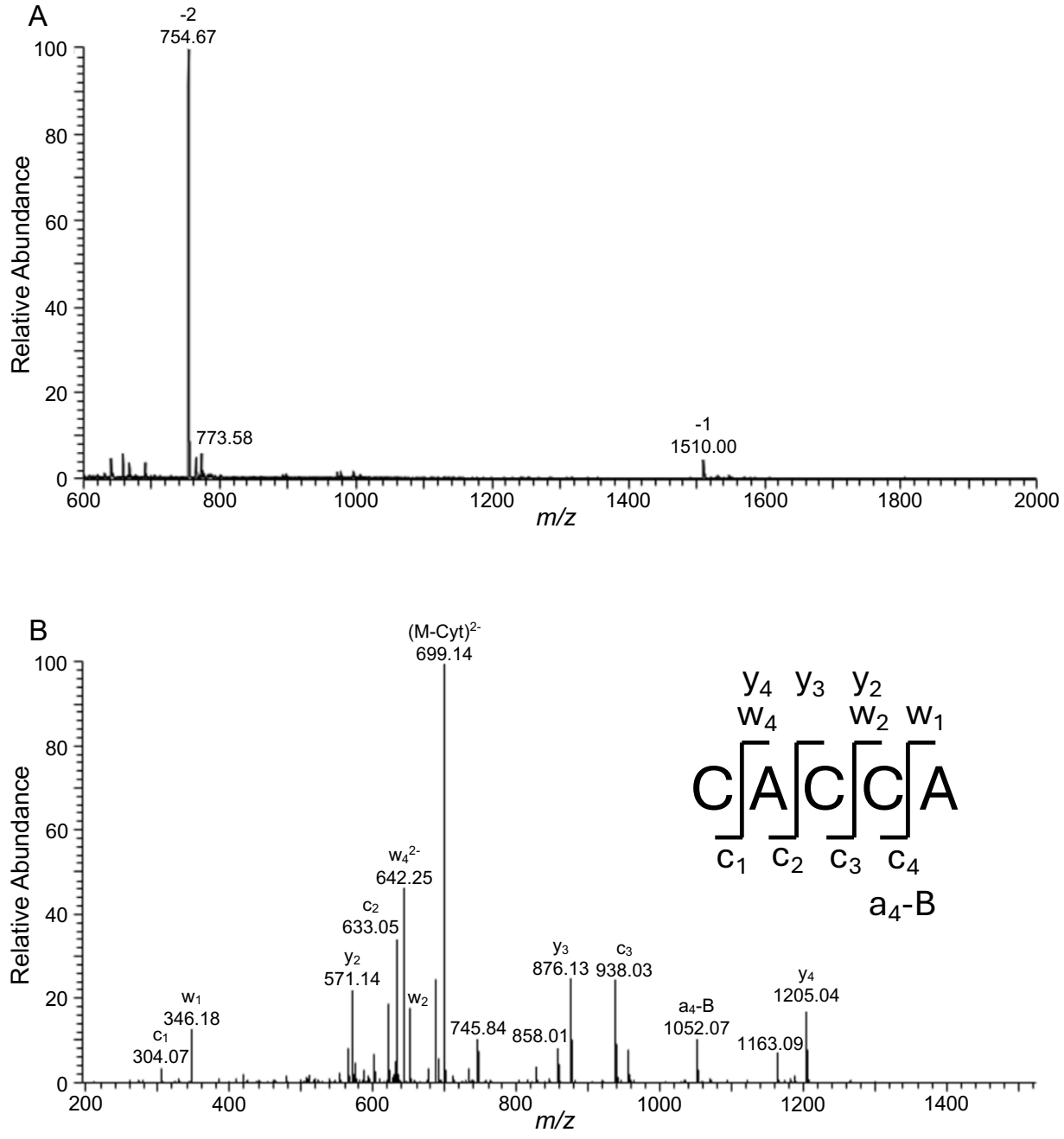
*CG and UG were below the scan range set at 600 *m/z*



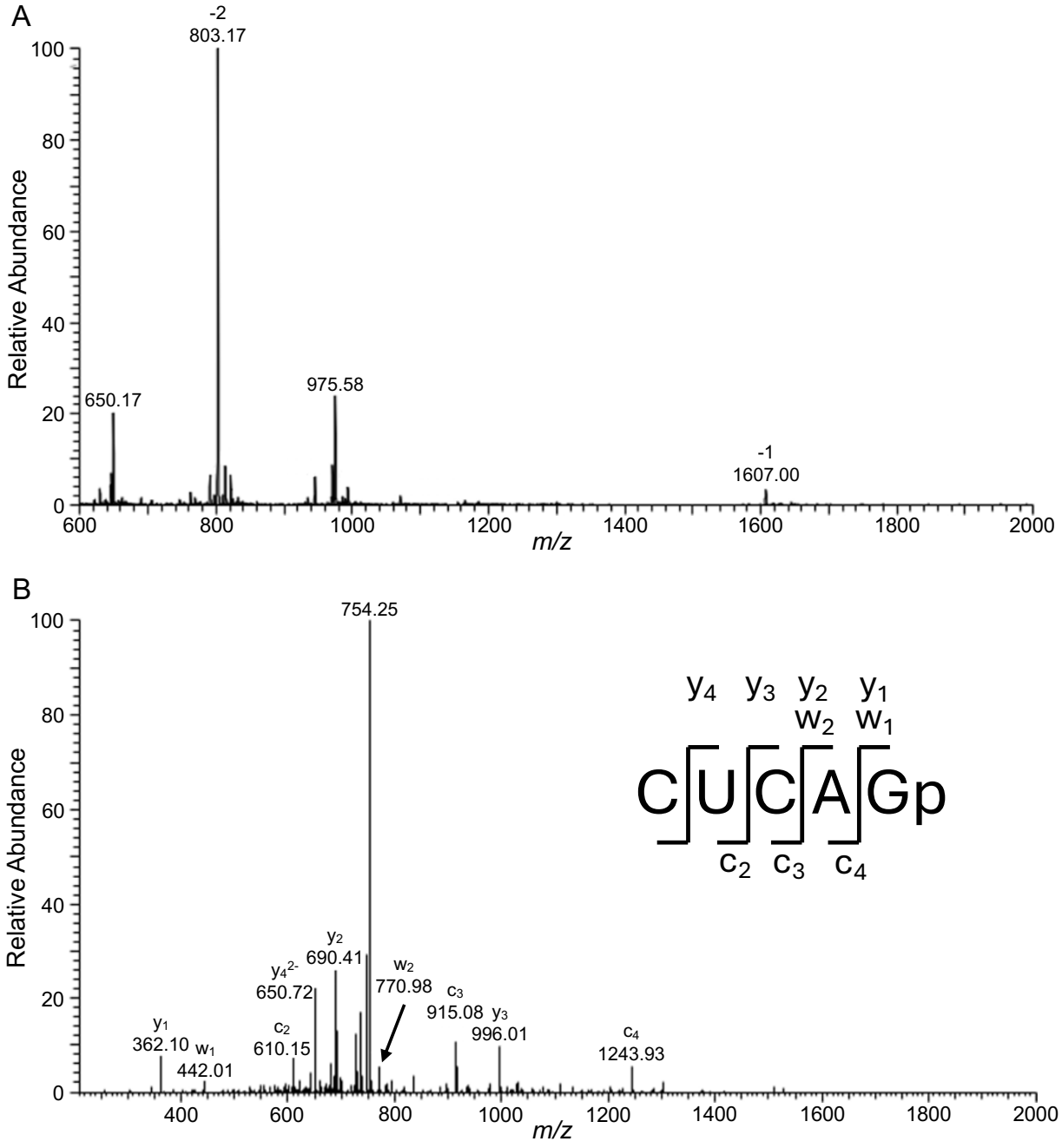
Supplemental Figure S1. Visual depiction of bonded phases of each column from Table 1. Dashed bonds represent a proprietary linker to affix the bonded phase to the particle. Blue particles are silica-based, and the pink particle is polymer-based.



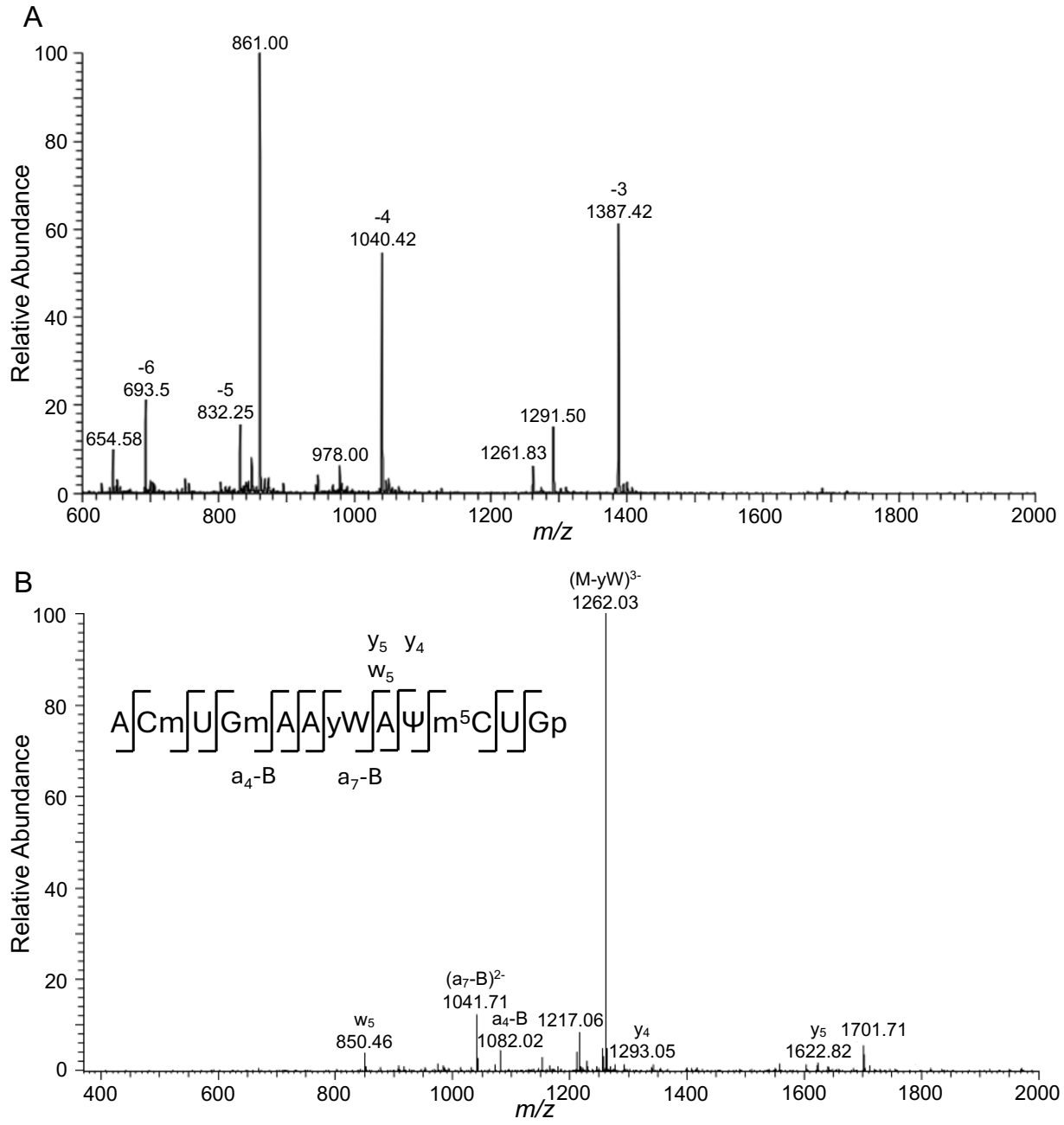
Supplemental Figure S2. LC-UV chromatograms showing separation of poly dTs by (**A**) Ace HILIC-N, (**B**) Waters BEH HILIC, (**C**) Waters Toros 2-pic, and (**D**) Waters Toros DEA columns. Under the conditions studied here, none of these alternative stationary phase chemistries are effective at the separation of longer oligonucleotides.



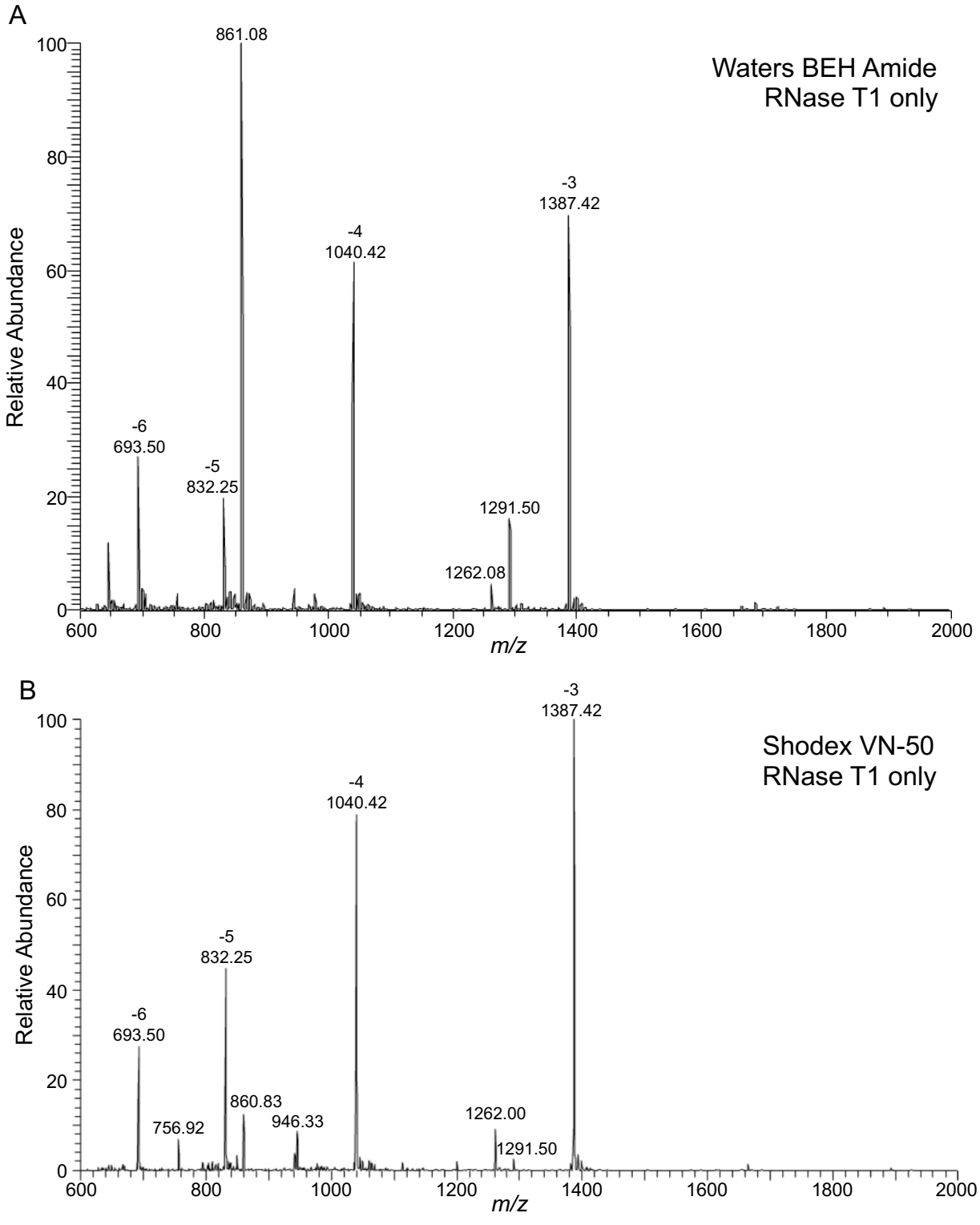
Supplemental Figure S3. (**A**) Mass spectrum of the RNase T1 digestion product CACCA, which elutes at 11.2 min from the Waters BEH Amide column (**Figure 5A**). (**B**) The MS/MS spectrum resulting from collision-induced dissociation of the -2 charge state. The fragmentation notation confirming this sequence is shown in the inset.

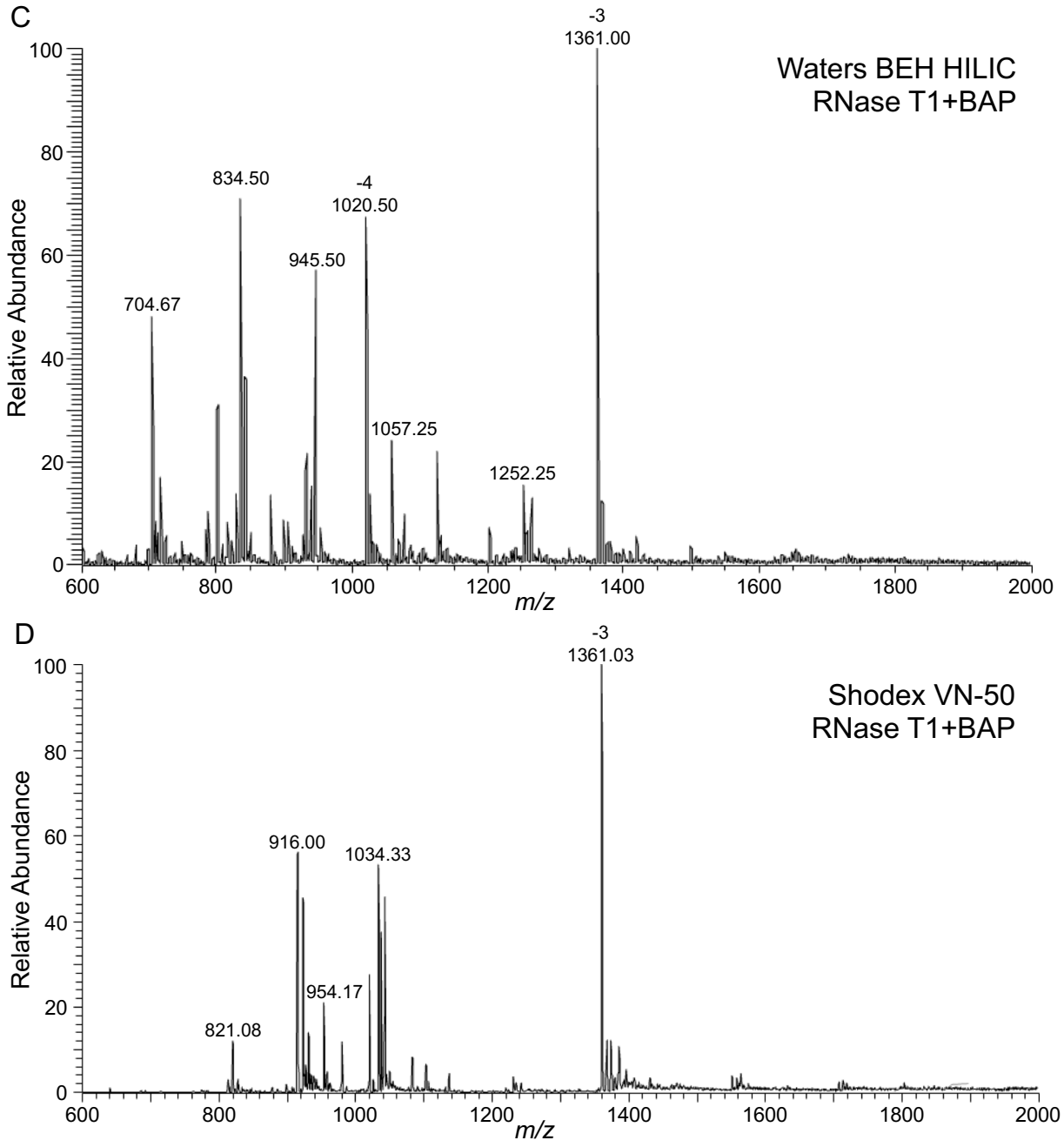


Supplemental Figure S4. (**A**) Mass spectrum of the RNase T1 digestion product CUCAGp, which elutes at 16 min from the Waters BEH Amide column (**Figure 5A**). (**B**) The MS/MS spectrum resulting from collision-induced dissociation of the -2 charge state. The fragmentation notation confirming this sequence is shown in the inset.



Supplemental Figure S5. (**A**) Mass spectrum of the RNase T1 digestion product A[Cm]U[Gm]AA[yW]A[Ψ][m^{5}C]UGp, which elutes at 20 min from the Waters BEH Amide column (**Figure 5A**). (**B**) The MS/MS spectrum resulting from collision-induced dissociation of the -3 charge state. The fragmentation notation assigned to this sequence is shown in the inset.


A
Waters BEH Amide
RNase T1 only
Relative Abundance
m/z
861.08
-3
1387.42
-4
1040.42
-6
693.50
-5
832.25
1291.50
1262.08
B
Shodex VN-50
RNase T1 only
Relative Abundance
m/z
-3
1387.42
-4
1040.42
-5
832.25
-6
693.50
860.83
946.33
756.92
1262.00
1291.50



Supplemental Figure S6. Representative mass spectra for the oligonucleotide A[Cm]U[Gm]AA[yW]A[Ψ][m^5C]UG(p) which is the largest RNase T1 digestion product from yeast tRNA-Phe. (**A**) Mass spectrum using the Waters BEH Amide column during RNase T1 digestion only. (**B**) Mass spectrum using the Shodex VN-50 column during RNase T1 digestion only. (**C**) Mass spectrum using the Waters BEH Amide column during RNase T1 and bacterial alkaline phosphatase (BAP) digestion. (**D**) Mass spectrum using the Shodex VN-50 column during RNase T1 and bacterial alkaline phosphatase (BAP) digestion. Addition of BAP apparently leads to significant salt adduction which negatively impacts the mass spectral results.