N-Terminal 4.0 Biphasic Column Protein Sequencer Methods

Technical Note

HP 241 Protein Sequencer (N+C)

The N-terminal 4.0 biphasic column sequencer methods implement the Edman degradation chemistry (coupling, cleavage, and conversion) on the Hewlett-Packard column-based protein sequencer. Reagents 1R, 2R, 3R, and the TH-Std are specific for C-terminal sequencing and are not utilized for the N-terminal 4.0 biphasic column sequencer methods.

These methods are suitable for N-terminal sequence analysis of most protein and peptide samples in the low nanomole to low picomole range which have been loaded onto a Hewlett-Packard biphasic RP/SAX column. The column should be pretreated with the Column prep 4.0 method prior to sample loading.

The methods are:
- N-Terminal Protein Column 4.0
- N-Terminal Protein Column 4.0 (Cycle 1)
- N-Terminal Peptide Column 4.0
- N-Terminal Peptide Column 4.0 (Cycle 1)
- N-Terminal Flask 4.0
- PTH-Std 4.0
- Column Prep 4.0

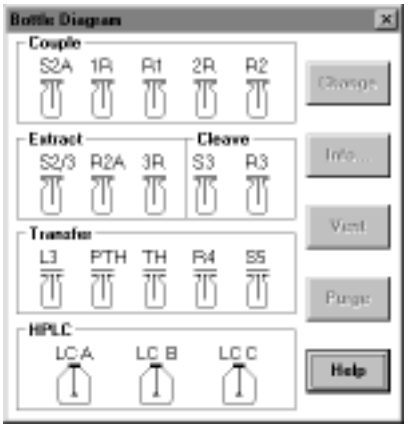
The column and flask methods control all of the derivatization/cleavage and conversion reactions, respectively.

The sequence program controls all of the column and flask methods, and well as any cycle exception methods. The N-terminal 4.0 biphasic column sequencer methods both use a cycle 1 column exception method.

The cycle 1 method provides a longer initial drying time and a pause to provide time for proper HPLC column equilibration after switching from C-terminal sequencing. This method also performs a double coupling on the N-terminal amino acid. There is no specialized cycle 1 method for the flask.

The PTH Std 4.0 method delivers 10 pmol/100 µl PTH-amino acid standard solution to the on-line HPLC allowing the quantitation and identification of sequenced amino acids. One may choose to run a PTH-standard by using PTH-std 4.0 when scheduling a sample.

The column prep 4.0 method is used to wash the sequencer column prior to sample loading. This has been found to be particularly important for low-level sample quantities (<10 pmol) samples.



The Routine 4.0 sequencer methods require the bottle configuration shown. The reagents and solvents are purchased from Hewlett-Packard. These methods produce less than 1mL/cycle of liquid waste.

S2A	Neat ethyl acetate
1R	Diphenylphosphorylisothiocyanate (DPP-ITC) in toluene/heptane (23:27:50)
R1	Phenylisothiocyanate (PITC) in heptane (3:97)
2R	Pyridine in ethylacetate (2:98)
R2	Diisopropylethylamine (DIEA) in 1-propanol/water (1:3:6)
S2/3	Acetonitrile/toluene (23:77)
R2A	Ocylamine in heptane (3:97)
3R	Potassium trimethylsilanolate (0.1M) in methanol/t-butanol (50:50)
S3	Acetonitrile/toluene (15:85)
R3	Neat trifluoroacetic acid (TFA)
L3	Acetic acid in methanol/water (1:74:25)
PTH	Mixture of PTH amino acids (10 pmol/100 µl) in acetonitrile with DPTU as a marker
TH	Mixture of TH amino acids (50 pmol/100 µl) in acetonitrile
R4	Trifluoroacetic acid (TFA) in water (1:3)
S5	Phosphate buffer (pH 2.9), 0.3% ion pairing reagent

Method Details

The steps for the N-terminal 4.0 biphasic column methods are described below. The steps indicate the ranges in volume that are appropriate for the various reagents/solvent deliveries. All sequencer methods are accessed by choosing the top menu item Edit/Method in the Protein Sequencer window. The sequencer column configuration used with the N-terminal 4.0 biphasic column methods consists of a reverse-phase (RP) sample column (top) mated with a strong anion exchange (SAX) column (bottom).

Method (Column): N-Terminal Protein Column 4.0

Total time: 45.8 min

All metering steps deliver the specified reagent or solvent to waste.

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9: The delivery of R1 should completely fill the column (both halves). The second R1 delivery may require a shorter meter time since the R1 bottle was already pressurized for the first delivery.

Step 12: The volume of R2 delivered by the end of Step 12 should wet about 1/3 to 1/2 of the way down the top reverse-phase (RP) column (approximately 2 slits distance as measured by the slits on the column wings). The bottom strong anion exchange (SAX) column should remain dry.

Step 13: The high pressure dry should deliver R2 about 2/3 of the way down the top RP column. The R2 should not wet the bottom SAX column.

Step 14: "Flush with S2A" flushes the delivery lines and should not wet the column

Steps 19, 21, 23, 25, 34, 38: The delivery of S2A should completely fill the column, followed by drying.

Step 29: During step 29, no more than to just below the upper frit of the top RP column should be wet with R3 (1 slit).

Step 32: After step 32, the volume of L3 delivered to the flask should be 50 µl (+/- 5 µl)

Step	Description	Primary Time	Temperature
1:	Couple: Dry column DOWN	60.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	4.5	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	4.3	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.0	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: Flush with S2A	15.0	55
15:	Couple: React	350.0	55
16:	Couple: Deliver from coupling bank DOWN	60.0	55
17:	Couple: Dry column UP	120.0	55
18:	Wash: Meter S2A	14.0	50
19:	Wash: Deliver and DRY DOWN (closed)	30.0	50
20:	Wash: Meter S2A	14.0	50
21:	Wash: Deliver and DRY DOWN (closed)	30.0	50
22:	Wash: Meter S2A	14.0	50
23:	Wash: Deliver and DRY DOWN (closed)	30.0	50
24:	Wash: Meter S2A	14.0	50
25:	Wash: Deliver and DRY DOWN (closed)	30.0	50
26:	Wash: Dry column DOWN	150.0	50
27	Cleave: Purge cleavage line		50
28:	Cleave: Meter R3	2.9	50
29:	Cleave: Deliver R3 DOWN	30.0	50
30:	Cleave: React	300.0	50
31:	!Cleave: Dry column DOWN to flask	60.0	50
32:	!Extract: Deliver L3 to flask	7.0	50
33:	!Extract: Meter S2A	14.0	50
34:	!Extract: Deliver solvent DOWN (closed)	15.0	50
35:	!Extract: Dry column DOWN to flask	5.0	50
36:	!Extract: Evaporate flask	50.0	50
37:	!Extract: Meter S2A	14.0	50
38:	!Extract: Deliver solvent DOWN (closed)	15.0	50
39:	!Extract: Dry column DOWN to flask	5.0	50
40:	!Extract: Evaporate flask	20.0	50
41:	Extract: Dry column DOWN to waste	60.0	55
42:	Couple: Dry column UP	60.0	55

Method (Column): N-Terminal Protein Column 4.0 (Cycle 1)
Total time: 76.3 min

Step 1: Cycle 1 begins with an extended dry to remove any residual solvent from the sample loading process. There is also a pause to provide time for proper HPLC column equilibration after switching from C-terminal sequencing.

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9, 18: The delivery of R1 should completely fill the column (both halves).). The second and third R1 delivery may require a shorter meter time since the R1 bottle was already pressurized for the first delivery.

Step 12, 21: The volume of R2 delivered by the end of Step 12 and 21 should wet about 1/3 to 1/2 of the way down the top reverse-phase (RP) column (approximately 2 slits distance as measured by the slits on the column wings). The bottom strong anion exchange (SAX) column should remain dry. The second R2 delivery may require a shorter meter time since the R2 bottle was already pressurized for the first delivery.

Step 13, 22: The high pressure dry should deliver R2 about 2/3 of the way down the top RP column. The R2 should not wet the bottom SAX column.

Step 23, 50: "Flush with S2A" flushes the delivery lines and should not wet the column

Steps 28, 30, 32, 34, 43, 47: These delivery of S2A should completely fill the column, followed by drying.

Step 38: During step 38, no more than to just below the upper frit of the top RP column should be wet with R3 (1 slit).

Step 41: After step 41, the volume of L3 delivered to the flask should be 50 µl (+/- 5 µl)

Step	Description	Primary Time	Temperature
1:	Couple: Dry column DOWN	300.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	4.5	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	4.3	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.0	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: React	200.0	55
15:	Couple: Deliver from coupling bank DOWN	60.0	55
16:	Couple: Dry column DOWN	60.0	55
17:	Couple: Meter R1	4.5	55
18:	Couple: Deliver R1 DOWN (closed)	20.0	55
19:	Couple: Dry column DOWN	60.0	55
20:	Couple: Meter R2	11.0	55
21:	Couple: Deliver R2 DOWN	20.0	55
22:	Couple: Dry column DOWN	60.0	55
23:	Couple: Flush with S2A	15.0	55
24:	Couple: React	200.0	55
25:	Couple: Deliver from coupling bank DOWN	60.0	55
26:	Couple: Dry column UP	120.0	55
27:	Wash: Meter S2A	14.0	50
28:	Wash: Deliver and DRY DOWN (closed)	30.0	50
29:	Wash: Meter S2A	14.0	50
30:	Wash: Deliver and DRY DOWN (closed)	30.0	50
31:	Wash: Meter S2A	14.0	50
32:	Wash: Deliver and DRY DOWN (closed)	30.0	50
33:	Wash: Meter S2A	14.0	50
34:	Wash: Deliver and DRY DOWN (closed)	30.0	50
35:	Wash: Dry column DOWN	150.0	50
36:	Wash: Purge solvent line		50
37:	Cleave: Meter R3	2.9	50
38:	Cleave: Deliver R3 DOWN	30.0	50
39:	Cleave: React	300.0	50
40:	!Cleave: Dry column DOWN to flask	60.0	50
41:	!Extract: Deliver L3 to flask	7.0	50
42:	!Extract: Meter S2A14.0	50	
43:	!Extract: Deliver solvent DOWN (closed)	15.0	50
44:	!Extract: Dry column DOWN to flask	5.0	50
45:	!Extract: Evaporate flask	50.0	50
46:	!Extract: Meter S2A	14.0	50
47:	!Extract: Deliver solvent DOWN (closed)	15.0	50
48:	!Extract: Dry column DOWN to flask	5.0	50
49:	!Extract: Evaporate flask	20.0	50
50:	Wash: Flush with S2A	15.0	55
51:	Extract: Dry column DOWN to waste	60.0	55
52:	Couple: Dry column UP	60.0	55

Method (Column): N-Terminal Peptide Column 4.0

Total time: 43.7 min

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9: The delivery of R1 should completely fill the column (both halves). The second R1 delivery may require a shorter meter time since the R1 bottle was already pressurized for the first delivery.

Step 12: The volume of R2 delivered by the end of Step 12 should wet about 1/3 to 1/2 of the way down the top reverse-phase (RP) column (approximately 2 slits distance as measured by the slits on the column wings). The bottom strong anion exchange (SAX) column should remain dry.

Step 13: The high pressure dry should deliver R2 about 2/3 of the way down the top RP column. The R2 should not wet the bottom SAX column.

Step 14, 39: "Flush with S2A" flushes the delivery lines and should not wet the column

Steps 19, 21, 31, 35: The delivery of S2/3 and S3 should completely fill the column, followed by drying.

Step 26: During step 26, no more than to just below the upper frit of the top RP column should be wet with R3 (1 slit).

Step 29: After step 29, the volume of L3 delivered to the flask should be 50 µl (+/- 5 µl)

Step	Description	Primary Time	Temperature
1:	Couple: Dry column DOWN	300.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	4.5	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	4.3	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.0	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: React	200.0	55
15:	Couple: Deliver from coupling bank DOWN	60.0	55
16:	Couple: Dry column DOWN	120.0	55
17:	Couple: Meter R1	4.5	55
18:	Couple: Deliver R1 DOWN (closed)	20.0	55
19:	Couple: Dry column DOWN	60.0	55
20:	Couple: Meter R2	11.0	55
21:	Couple: Deliver R2 DOWN	20.0	55
22:	Couple: Dry column DOWN	60.0	55
23:	Couple: Flush with S2A	15.0	55
24:	Couple: React	200.0	55
25:	Couple: Deliver from coupling bank DOWN	60.0	55
26:	Couple: Dry column UP	120.0	55
27:	Wash: Purge solvent line		50
28:	Wash: Meter S2/3	17.0	50
29:	Wash:Deliver and DRY DOWN (closed)	30.0	50
30:	Wash: Meter S2/3	17.0	50
31:	Wash:Deliver and DRY DOWN (closed)	30.0	50
32:	Wash: Meter S2/3	17.0	50
33:	Wash:Deliver and DRY DOWN (closed)	30.0	50
34:	Wash: Meter S2/3	17.0	50
35:	Wash:Deliver and DRY DOWN (closed)	30.0	50
36:	Wash: Dry column DOWN	120.0	50
37:	Cleave: Purge cleavage line		50
38:	Cleave: Meter R3	2.9	50
39:	Cleave: Deliver R3 DOWN with dry	30.0	50
40:	Cleave: React	300.0	50
41:	!Cleave: Dry column DOWN to flask	60.0	50
42:	!Extract: Deliver L3 to flask	7.0	50
43:	!Extract: Meter S3	17.0	50
44:	!Extract: Deliver solvent DOWN (closed)	10.0	50
45	!Extract: Dry column DOWN to flask	5.0	50
46:	!Extract: Evaporate flask	50.0	50
47:	!Extract: Meter S2/3	17.0	50
48:	!Extract: Deliver solvent DOWN (closed)	10.0	50
49:	!Extract: Dry column DOWN to flask	5.0	50
50:	!Extract: Evaporate flask	20.0	50
51:	Extract: Dry column DOWN to waste	60.0	50
52:	Wash: Flush with S2A	15.0	55
53:	Wash: Dry column UP	120.0	55

Method (Column): N-Terminal Peptide Column 4.0 (Cycle 1)
Total time: 77.1 min

Step 1: Cycle 1 begins with an extended dry to remove any residual solvent from the sample loading process. There is also a pause to provide time for proper HPLC column equilibration after switching from C-terminal sequencing.

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9, 18: The delivery of R1 should completely fill the column (both halves). The second and third R1 delivery may require a shorter meter time since the R1 bottle was already pressurized for the first delivery.

Step 12, 21: The volume of R2 delivered by the end of Step 12 and 21 should wet about 1/3 to 1/2 of the way down the top reverse-phase (RP) column (approximately 2 slits distance as measured by the slits on the column wings). The bottom strong anion exchange (SAX) column should remain dry. The second R2 delivery may require a shorter meter time since the R2 bottle was already pressurized for the first delivery.

Step 13, 22: The high pressure dry should deliver R2 about 2/3 of the way down the top RP column. The R2 should not wet the bottom SAX column.

Step 23, 50: "Flush with S2A" flushes the delivery lines and should not wet the column

Steps 29, 31, 33, 35, 44, 48: The delivery of S2/3 and S3 should completely fill the column, followed by drying.

Step 39: During step 39, no more than to just below the upper frit of the top RP column should be wet with R3 (1 slit).

Step 42: After step 42, the volume of L3 delivered to the flask should be 50 µl (+/- 5 µl)

Step	Description	Primary Time	Temperature
1:	Couple: Dry column DOWN	300.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	4.5	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	4.3	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.0	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: React	200.0	55
15:	Couple: Deliver from coupling bank DOWN	60.0	55
16:	Couple: Dry column DOWN	120.0	55
17:	Couple: Meter R1	4.5	55
18:	Couple: Deliver R1 DOWN (closed)	20.0	55
19:	Couple: Dry column DOWN	60.0	55
20:	Couple: Meter R2	11.0	55
21:	Couple: Deliver R2 DOWN	20.0	55
22:	Couple: Dry column DOWN	60.0	55
23:	Couple: Flush with S2A	15.0	55
24:	Couple: React	200.0	55
25:	Couple: Deliver from coupling bank DOWN	60.0	55
26:	Couple: Dry column UP	120.0	55
27:	Wash: Purge solvent line		50
28:	Wash: Meter S2/3	17.0	50
29:	Wash:Deliver and DRY DOWN (closed)	30.0	50
30:	Wash: Meter S2/3	17.0	50
31:	Wash:Deliver and DRY DOWN (closed)	30.0	50
32:	Wash: Meter S2/3	17.0	50
33:	Wash:Deliver and DRY DOWN (closed)	30.0	50
34:	Wash: Meter S2/3	17.0	50
35:	Wash:Deliver and DRY DOWN (closed)	30.0	50
36:	Wash: Dry column DOWN	120.0	50
37:	Cleave: Purge cleavage line		50
38:	Cleave: Meter R3	2.9	50
39:	Cleave: Deliver R3 DOWN with dry	30.0	50
40:	Cleave: React	300.0	50
41:	!Cleave: Dry column DOWN to flask	60.0	50
42:	!Extract: Deliver L3 to flask	7.0	50
43:	!Extract: Meter S3	17.0	50
44:	!Extract: Deliver solvent DOWN (closed)	10.0	50
45	!Extract: Dry column DOWN to flask	5.0	50
46:	!Extract: Evaporate flask	50.0	50
47:	!Extract: Meter S2/3	17.0	50
48:	!Extract: Deliver solvent DOWN (closed)	10.0	50
49:	!Extract: Dry column DOWN to flask	5.0	50
50:	!Extract: Evaporate flask	20.0	50
51:	Extract: Dry column DOWN to waste	60.0	50
52:	Wash: Flush with S2A	15.0	55
53:	Wash: Dry column UP	120.0	55

Method (Flask): N-Terminal Flask 4.0

Total time: 34.6 min

Step 1: During Step 1, the liquid in the flask partially evaporates but must not dry down completely (10 - 20 µl remains). The flask will continue to dry during the first part of Step 2.

Step 2: During Step 2, the volume of R4 delivered to the flask should be approximately 70 µl (+/- 5 µl)

Step 4: By the end of Step 4, the flask will have been dry for 100-150 seconds.

Step 5: By the end of Step 5, 70 µl (+/- 5 µl) of L3 will have been delivered to the flask

Step 6: By the end of Step 6, the flask will have been dry for 75-100 seconds

Step 7: The delivery of L3 may not be visible

Step 8: After Step 8, the volume of L3 and S5 in the flask should be 75-80 µl. If adjustment is needed, it should be done by adjusting the metering time of S5, leaving L3 unchanged.

TEST Delivery protocol:
To check the delivery volume of L3 and S5, copy steps 7, 8, 9, and 10 to the clipboard and run from the clipboard. At the end of Step 10, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that some of the delivered volume is not retained on the tube sides or top of the flask.

Step 11: Before injection the injector loop should be filled, leaving the solvent visible in both the inlet and outlet lines of the injector loop. The time for Step 11 may have to be adjusted in order to optimize the delivery.

Step	Description	Primary Time	Temperature
1:	Convert: Evaporate	40.0	70
2:	Convert: Deliver R4	29.0	70
3:	Convert: React	400.0	70
4:	Convert: Evaporate dry	400.0	70
5:	Convert: Flush with L3	15.0	70
6:	Convert: Evaporate dry L3	140.0	70
7:	Convert: Deliver L3 dripwise	2.0	60
8:	Convert: Deliver S5 dripwise	12.0	60
9:	Convert: Mix and equilibrate	15.0	60
10:	Convert: Solubilize	20.0	60
11:	Convert: Fill loop and inject	2.0	60
12:	Convert: Empty flask	30.0	60
13:	Convert: Flush with L3	85.0	45
14:	Convert: Mix	20.0	45
15:	Convert: Empty flask	30.0	45
16:	Convert: Deliver R4 wash	40.0	45
17:	Convert: Empty flask	30.0	45
18:	Convert: Deliver R4 wash	0.0	45
19:	Convert: Empty flask	30.0	45
20:	Convert: Dry waste line	60.0	45
21:	Convert: Dry vent line	60.0	45

Method (Flask): PTH Std 4.0
Total time: 50.3 min

This PTH Std 4.0 method is appropriate with any of the N-terminal sequence programs. Ten picomoles of PTH standard are delivered to the flask in this method.

Step 1: Step 1: Cycle 1 begins with a pause to provide time for proper HPLC column equilibration after switching from C-terminal sequencing.

Step 4: Step 4 should add 100 µl (+/- 2 µl) of PTH-standard to the dry flask

TEST Delivery protocol:
To check the delivery volume of the PTH-standards, copy the steps in the PTH Std Test 4.0 method to the clipboard and run from the clipboard. At the end of test program, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that some of the delivered volume is not retained on the tube sides or top of the flask

Step 6: The delivery of L3 may not be visible

Step 7: After Step 7, the volume of L3 and S5 in the flask should be 75-80 µl. If adjustment is needed, it should be done by adjusting the metering time of S5, leaving L3 unchanged.

TEST Delivery protocol:
To check the delivery volume of L3 and S5, copy steps 6, 7, 8, and 9 to the clipboard and run from the clipboard. At the end of Step 9, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that some of the delivered volume is not retained on the tube sides or top of the flask

Step 10: Before injection the injector loop should be filled, leaving the solvent visible in both the inlet and outlet lines of the injector loop. The time for Step 10 may have to be adjusted in order to optimize the delivery.

Step	Description	Primary Time	Temperature
1:	Convert: Empty flask	120.0	60
2:	Convert: Dry waste line	200.0	60
3:	Convert: Dry vent line	200.0	60
4:	Convert: Deliver PTH Std with L3 purge	6.9	60
5:	Convert: Evaporate dry standard	180.0	60
6:	Convert: Deliver L3 dripwise	2.0	60
7:	Convert: Deliver S5 dripwise	12.0	60
8:	Convert: Mix and equilibrate	15.0	60
9:	Convert: Solubilize	20.0	60
10:	Convert: Fill loop and inject	2.0	60
11:	Convert: Empty flask	30.0	60
12:	Convert: Flush with L3	120.0	45
13:	Convert: Mix	20.0	45
14:	Convert: Empty flask	30.0	45
15:	Convert: Deliver R4 wash	40.0	45
16:	Convert: Empty flask	30.0	45
17:	Convert: Deliver R4 wash	40.0	45
18:	Convert: Empty flask	30.0	45
19:	Convert: Dry waste line	250.0	45
20:	Convert: Dry vent line	250.0	45

Method (Column): Column Prep 4.0

Total time: 19.5 min

Steps 3, 14, 20, 23, 25, 27, 29: The delivery of S2A and S2/3 should completely fill the column, followed by drying.

Steps 6, 17: The delivery of R2A and R3 should completely fill the column (both halves)

Step 9: The delivery of R2 in Step 9 should completely fill the column (both halves)

Step	Description	Primary Time	Temperature
1:	Col prep: Purge coupling line	8.0	60
2:	Col prep: Meter S2A	12.0	60
3:	Col prep: Deliver from coupling bank DOWN	15.0	60
4:	Col prep: Dry column DOWN	10.0	60
5:	Col prep: Meter R2A	10.0	60
6:	Col prep: Deliver R2A DOWN (closed)	20.0	60
7:	Col prep: Dry column DOWN	30.0	60
8:	Col prep: Meter R2	50.0	60
9:	Col prep: Deliver R2 DOWN (closed)	45.0	60
10:	Col prep: wait	90.0	60
11:	Col prep: Dry column DOWN to waste	60.0	60
12:	Col prep: Purge coupling line	30.0	60
13:	Col prep: Meter S2A	12.0	60
14:	Col prep: Deliver from coupling bank DOWN	15.0	60
15:	Col prep: Dry column DOWN	120.0	60
16:	Col prep: Meter R3	15.0	60
17:	Col prep: Deliver from cleavage bank DOWN		60
18:	Col prep: Dry column DOWN	10.0	60
19:	Col prep: Meter S2A	12.0	60
20:	Col prep: Deliver from coupling bank DOWN	15.0	60
21:	Col prep: Dry column DOWN	60.0	60
22:	Col prep: Meter S2/3	17.0	60
23:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
24:	Col prep: Meter S2/3	17.0	60
25:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
26:	Col prep: Meter S2/3	17.0	60
27:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
28:	Col prep: Meter S2/3	17.0	60
29:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
30:	Col prep: Dry column DOWN	60.0	60

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