Sequoia Sciences

High-throughput Natural Products Chemistry

Where Did My Compound Go?

Purifying Small Amounts

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Common Problem: You ALWAYS want more material

- Different challenges at different scales
- Today's focus: 10 µg – 1 mg purified compound

General Strategies for Small Amounts

- Limit transfer steps
  - And use appropriately sized containers
- Move to HPLC as soon as possible
  - Early steps may vary
  - Single round of semi-preparative HPLC
- Rethink quantitation

Pre-HPLC steps: an example

Extract →

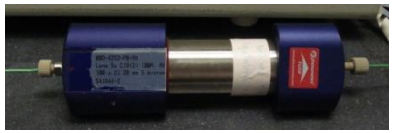
Flash chromatography

- Normal phase
- 1 g portions
- 50 g column
- 5 fractions

→

Rotovap

→



Prep HPLC

- Reversed phase
- 50 mg portions
- 21 x 100 mM column
- 40 fractions

→ Deep-well plate

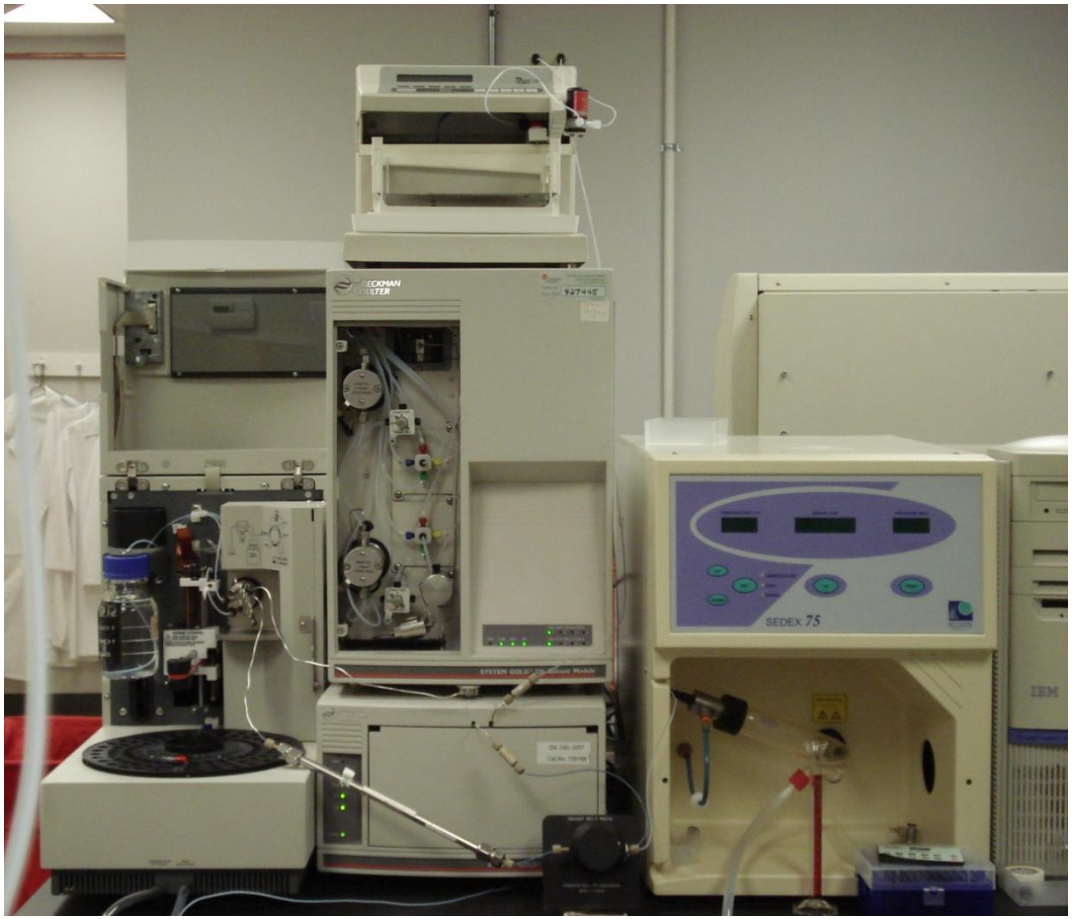
- Quantitation
- Bioassay

Active fractions to HPLC vial

- May include multiple runs
- Also consider neighbor wells

HPLC System

- Injector
- Column
- Detector
- Fraction collector



HPLC Injector

- Manual
  - Choose appropriate loop size
- Autoinjector
  - Read the manual; set for full recovery mode

HPLC Columns

- Analytical/semipreparative
  - 250 mm length
  - 4.6-10 mm width
- Solid phase choices
  - We use a variety of reversed phase, mostly C18
  - Same column for “scouting” and collection

Developing an HPLC method

- Goal: pure compounds from single pass
- Use same column/system for method development and collection
- Choose initial conditions based on what you know about polarity from previous steps
- Balancing act: inject enough to see the peaks, without blowing all your material

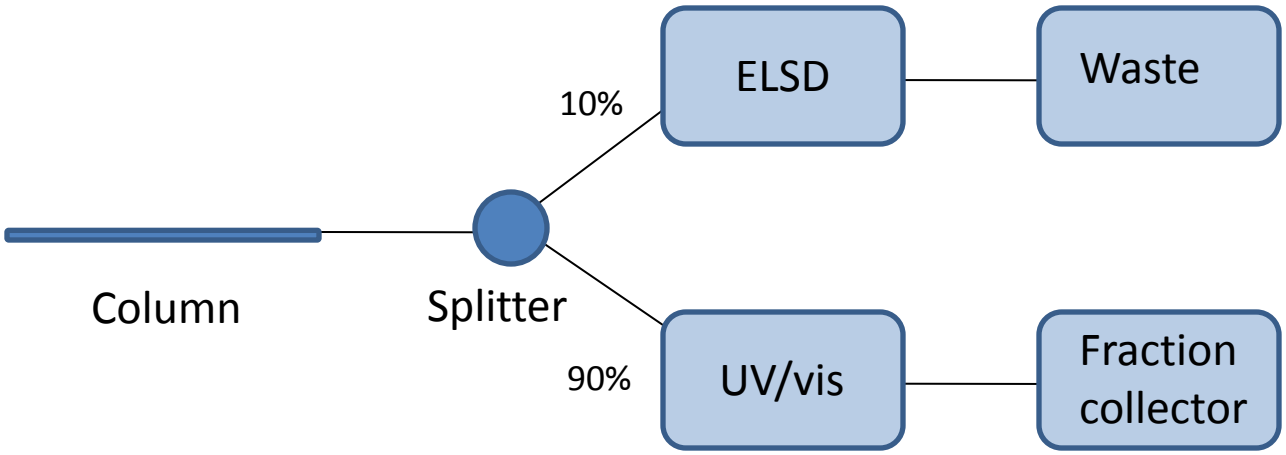
Developing an HPLC method, cont.

1) Find a “good enough” method (1-3 injections)
2) Use this method to scout other relevant fractions
3) Pool all relevant fractions
4) Can the method be made better?
    1) Modify gradient to stretch chromatogram
    2) If blobby clusters, consider a different column
5) Collect pool in as few injections possible while maintaining resolution

HPLC Detection: How to detect and still collect

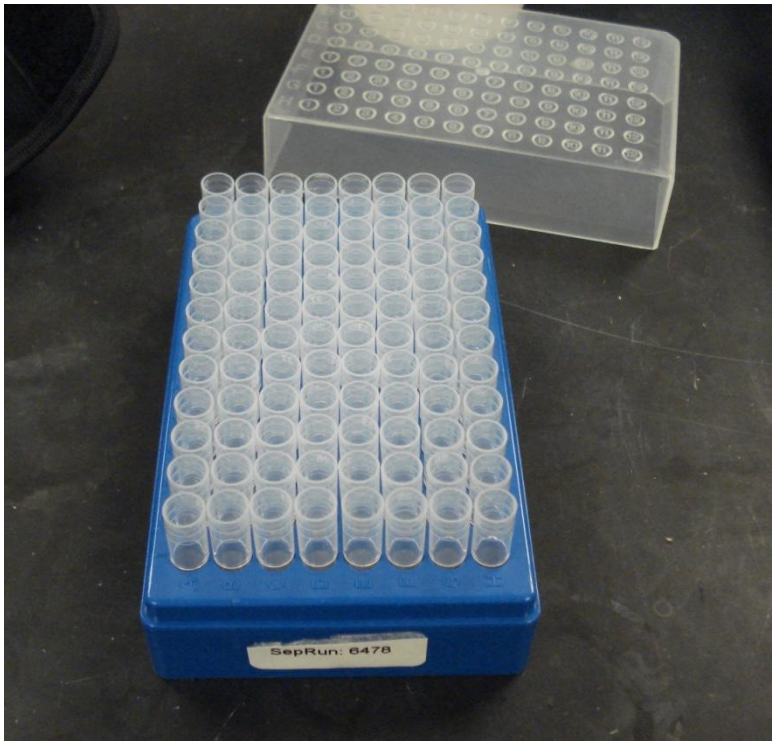
- UV/vis
  - Non-destructive
  - Requires a chromophore (not so good for unknowns)
  - Not quantitative for unknowns
- ELSD (Evaporative Light Scattering)
  - Destructive
  - Detects nearly all compounds
  - Quantitative (can use for quantitation with standard curve of model compounds)
    - Log area = m(log amount) + b

How to detect and still collect: the splitter

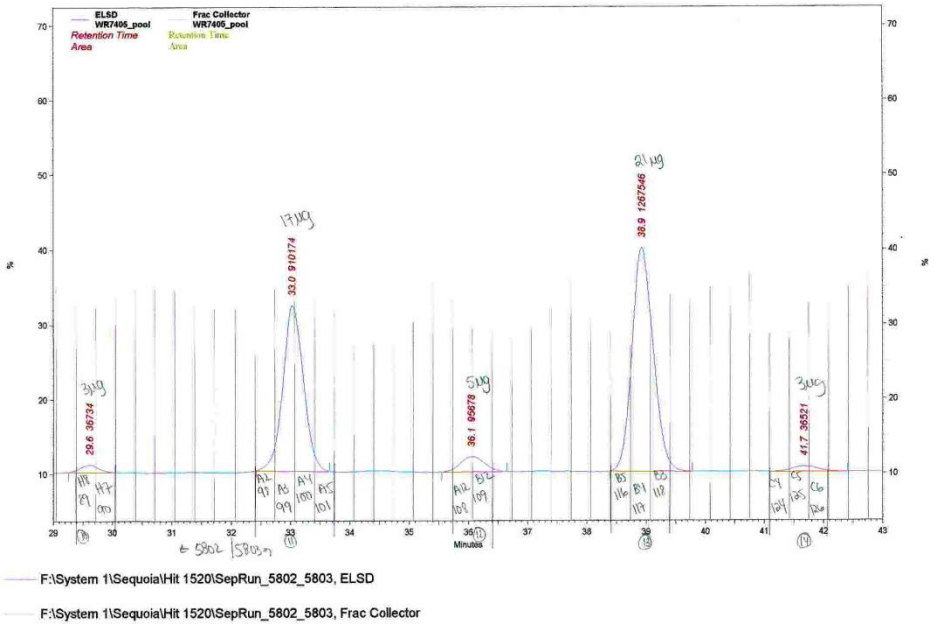


Fraction Collector

- 96-well format
- Speed-vac compatible
- “Minitubes” useful for pooling
- Marks on chromatogram
- Need to know which fraction is which



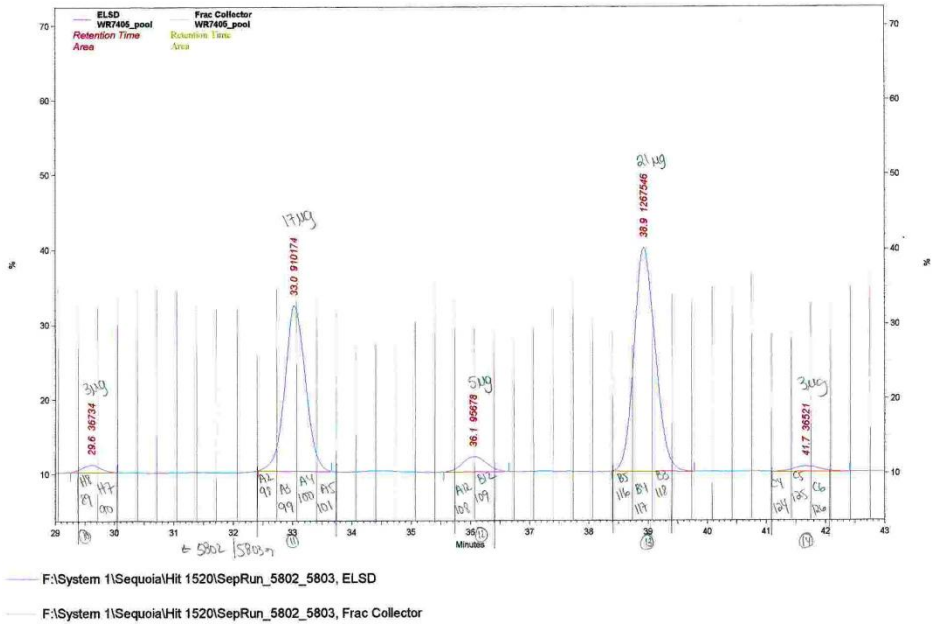
Pooling fractions-on paper



Appendix B
HPLC System #1
Foxy Jr. Fraction Collector (SN 200G20074)
Program 3: 10 Minute Collection Delay
(Validation DEV-031)

Sep Well#	Plate Position	Well End Time (Minutes)	Sep Well#	Plate Position	Well End Time (Minutes)	Sep Well#	Plate Position	Well End Time (Minutes)
1	A1	10.33	33	C9	21.00	65	F8	31.67
2	A2	10.67	34	C10	21.33	66	F7	32.00
3	A3	11.00	35	C11	21.67	67	F6	32.33
4	A4	11.33	36	C12	22.00	68	F5	32.67
5	A5	11.67	37	D12	22.33	69	F4	33.00
6	A6	12.00	38	D11	22.67	70	F3	33.33
7	A7	12.33	39	D10	23.00	71	F2	33.67
8	A8	12.67	40	D9	23.33	72	F1	34.00
9	A9	13.00	41	D8	23.67	73	G1	34.33
10	A10	13.33	42	D7	24.00	74	G2	34.67
11	A11	13.67	43	D6	24.33	75	G3	35.00
12	A12	14.00	44	D5	24.67	76	G4	35.33
13	B12	14.33	45	D4	25.00	77	G5	35.67
14	B11	14.67	46	D3	25.33	78	G6	36.00
15	B10	15.00	47	D2	25.67	79	G7	36.33
16	B9	15.33	48	D1	26.00	80	G8	36.67
17	B8	15.67	49	E1	26.33	81	G9	37.00
18	B7	16.00	50	E2	26.67	82	G10	37.33
19	B6	16.33	51	E3	27.00	83	G11	37.67
20	B5	16.67	52	E4	27.33	84	G12	38.00
21	B4	17.00	53	E5	27.67	85	H12	38.33
22	B3	17.33	54	E6	28.00	86	H11	38.67
23	B2	17.67	55	E7	28.33	87	H10	39.00
24	B1	18.00	56	E8	28.67	88	H9	39.33
25	C1	18.33	57	E9	29.00	89	H8	39.67
26	C2	18.67	58	E10	29.33	90	H7	40.00
27	C3	19.00	59	E11	29.67	91	H6	40.33
28	C4	19.33	60	E12	30.00	92	H5	40.67
29	C5	19.67	61	F12	30.33	93	H4	41.00
30	C6	20.00	62	F11	30.67	94	H3	41.33
31	C7	20.33	63	F10	31.00	95	H2	41.67
32	C8	20.67	64	F9	31.33	96	H1	42.00

Pooling fractions-on paper



Hit 1520 WR 7405
CS 8-16-11
NMR & Screening

Peak Number	SepRun	SepWell	Peak Designation by ELSD	SepFraction	Real Amount upon creation (ug)	ELSD total (ug)
1	5802	22-23	B3-B2	19618	2	
1	5804	22-23	B3-B2	19618	2	
1	5810	22-23	B3-B2	19618	2	
1	5812	22-23	B3-B2	19618	2	8
2	5802	28-29	C4-C5	19619	12	
2	5804	28-29	C4-C5	19619	12	
2	5810	28-29	C4-C5	19619	12	
2	5812	28-29	C4-C5	19619	13	49
3	5802	39-40	D10-D9	19620	2	
3	5804	40	D9	19620	2	
3	5810	40	D9	19620	1	
3	5812	40	D9	19620	1	6
4	5802	42	D7	19621	3	
4	5804	42	D7	19621	3	
4	5810	42	D7	19621	3	
4	5812	42-43	D7-D6	19621	3	12
5	5802	43-44	D6-D5	19622	5	
5	5804	44	D5	19622	5	
5	5810	44-45	D5-D4	19622	6	
5	5812	44-45	D5-D4	19622	6	22
6	5802	53-55	E5-E7	19623	13	
6	5804	53-55	E5-E7	19623	13	
6	5810	54-55	E6-E7	19623	13	
6	5812	54-55	E6-E7	19623	15	54
7	5802	56-57	E8-E9	19624	4	
7	5804	56-57	E8-E9	19624	4	
7	5810	57-58	E9-E10	19624	4	
7	5812	57-58	E9-E10	19624	4	16
8	5802	60-61	E12; F12	19625	18	
8	5804	60-61	E12; F12	19625	17	
8	5810	60-62	E12; F12-F11	19625	18	
8	5812	61-62	F12-F11	19625	19	72
9	5802	65-66	F8-F7	19626	2	
9	5804	66	F7	19626	1	
9	5810	66-67	F7-F6	19626	1	
9	5812	66-67	F7-F6	19626	1	5
10	5802	89-90	H8-H7	19627	3	
10	5804	89-90	H8-H7	19627	2	
10	5810	90-91	H7-H6	19627	2	
10	5812	90-91	H7-H6	19627	3	10
11	5803	98-101	A2-A5	19628	17	
11	5805	99-101	A3-A5	19628	17	
11	5811	100-102	A4-A6	19628	17	
11	5813	100-102	A4-A6	19628	18	69

Pooling fractions-in the world

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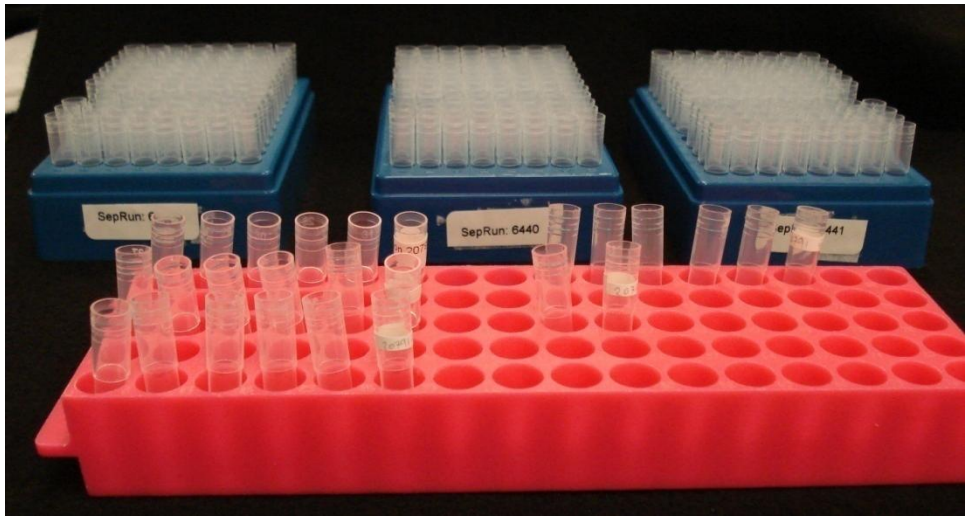
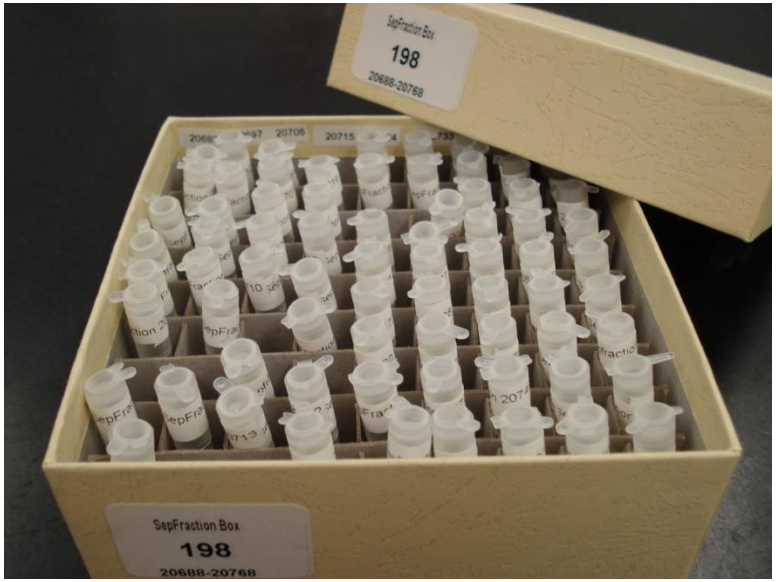
1	5810	22-23	B3-B2	19618	2	
1	5812	22-23	B3-B2	19618	2	8
2	5802	28-29	C4-C5	19619	12	
2	5804	28-29	C4-C5	19619	12	
2	5810	28-29	C4-C5	19619	12	
2	5812	28-29	C4-C5	19619	13	49
3	5802	39-40	D10-D9	19620	2	
3	5804	40	D9	19620	2	
3	5810	40	D9	19620	1	
3	5812	40	D9	19620	1	6
4	5802	42	D7	19621	3	
4	5804	42	D7	19621	3	
4	5810	42	D7	19621	3	
4	5812	42-43	D7-D6	19621	3	12
5	5802	43-44	D6-D5	19622	5	
5	5804	44	D5	19622	5	
5	5810	44-45	D5-D4	19622	6	
5	5812	44-45	D5-D4	19622	6	22
6	5802	53-55	E5-E7	19623	13	
6	5804	53-55	E5-E7	19623	13	
6	5810	54-55	E6-E7	19623	13	
6	5812	54-55	E6-E7	19623	15	54
7	5802	56-57	E8-E9	19624	4	
7	5804	56-57	E8-E9	19624	4	
7	5810	57-58	E9-E10	19624	4	
7	5812	57-58	E9-E10	19624	4	16
8	5802	60-61	E12; F12	19625	18	
8	5804	60-61	E12; F12	19625	17	
8	5810	60-62	E12; F12-F11	19625	18	
8	5812	61-62	F12-F11	19625	19	72
9	5802	65-66	F8-F7	19626	2	
9	5804	66	F7	19626	1	
9	5810	66-67	F7-F6	19626	1	
9	5812	66-67	F7-F6	19626	1	5
10	5802	89-90	H8-H7	19627	3	
10	5804	89-90	H8-H7	19627	2	
10	5810	90-91	H7-H6	19627	2	
10	5812	90-91	H7-H6	19627	3	10
11	5803	98-101	A2-A5	19628	17	
11	5805	99-101	A3-A5	19628	17	
11	5811	100-102	A4-A6	19628	17	
11	5813	100-102	A4-A6	19628	18	69



Pooling fractions-in the world



Easy to retrieve and dissolve for bioassay, NMR, etc.

Strategies for Small Amounts-Summary

- Limit transfer steps
- Limit purification steps
- Match equipment/supply size to amounts
- Rethink quantitation
- Be organized and methodical