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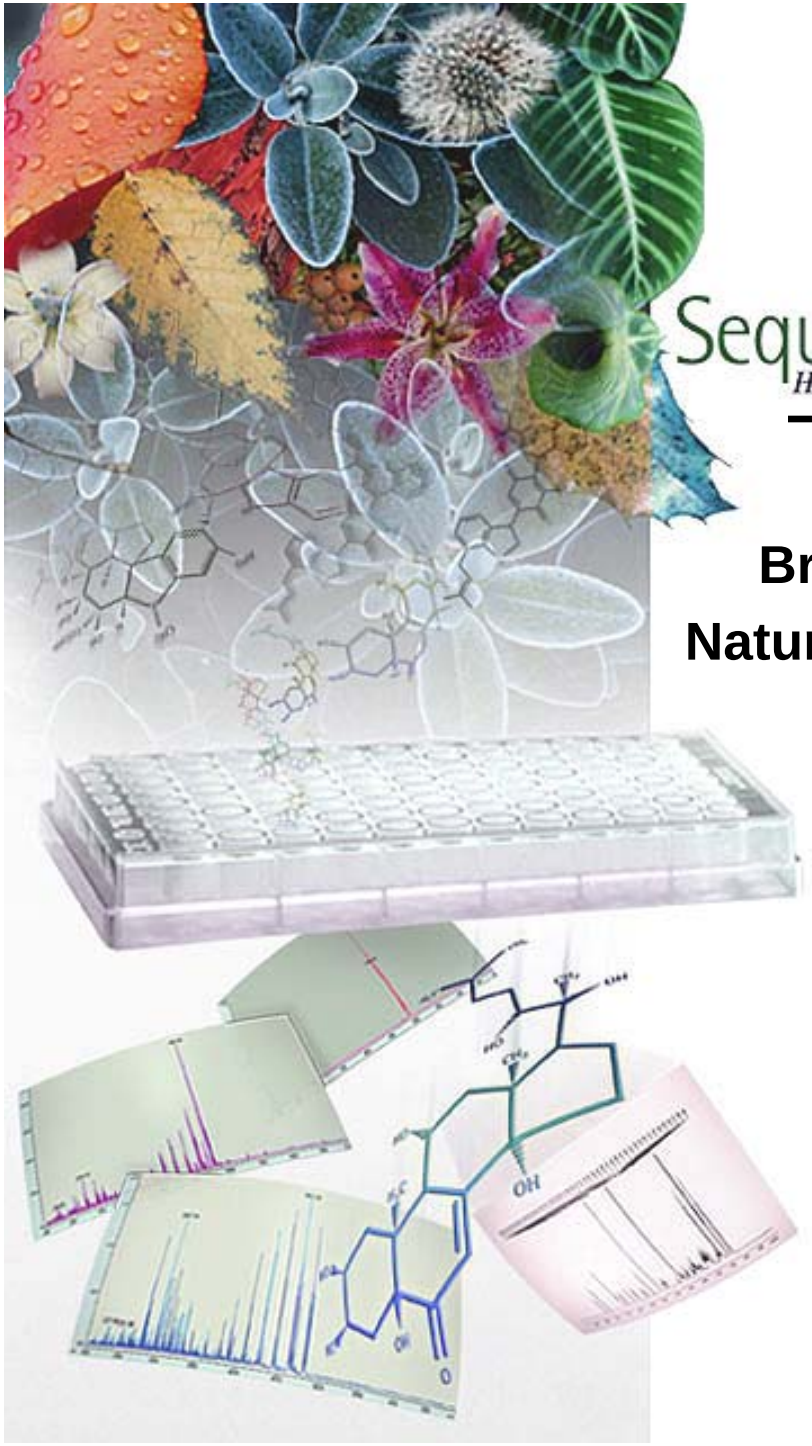

Sequoia Sciences
High-throughput Natural Products Chemistry

Bringing Back Nature to Drug Discovery
Natural Molecules in an Antibacterial Program

Mark O'Neil-Johnson

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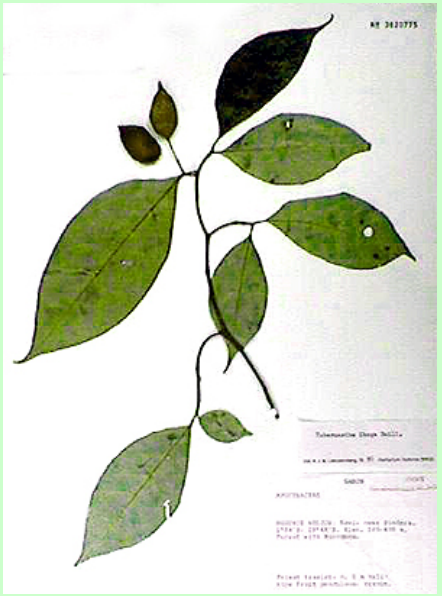
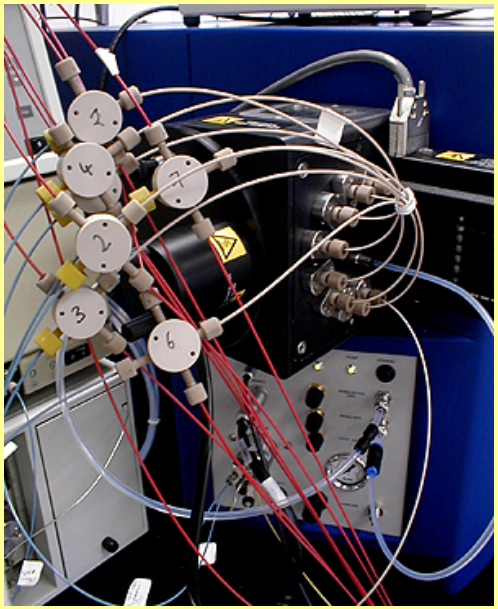
High-throughput Natural Products Chemistry

Sequoia Sciences, Inc.



Taxonomically Diverse Plant Collection



Parallel Purification Technologies

State-of-the-art NMR Probe Technology

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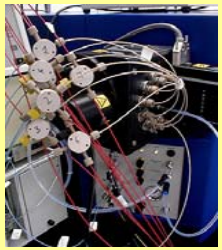
Plants to Compound Library

① Sequoia's Strengths

Taxonomically Diverse Plant Collection



Parallel Purification Technologies

State-of-the-art NMR Probe Technology

② Five step purification process to remove non-drug-like compounds

- Approximately 90% of plant extracts are composed of "non-drug-like" compounds

Highly Lipophilic Compounds	> Log P of 5
Highly Hydrophilic Compounds	< Log P of 0
High Molecular Weight Compounds	> 1000 Daltons
Miscellaneous Compounds	
i.e. Carbohydrates, Tannins	

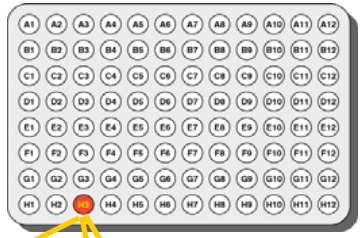
- Sequoia's process removes these "non-drug-like" compounds
- Generates approximately 110 preparative HPLC fractions ("sample") per plant

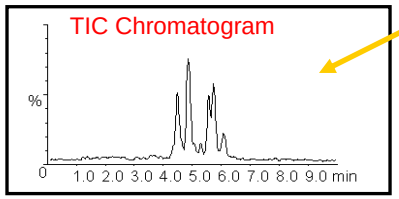
③ Analysis performed on each sample

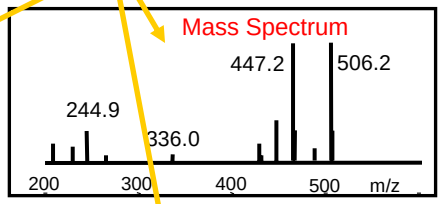
8-channel MUX LC/MS with 8 ELSD's

~ 110 samples / plant

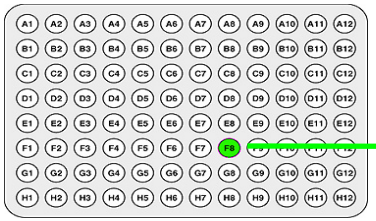
1. **1 to 5 compounds per sample**
2. **Samples of known quantities**
3. **Drug-like compounds**
   a. **Average MW < 400 daltons**
   b. **Log P from 0 to 5**

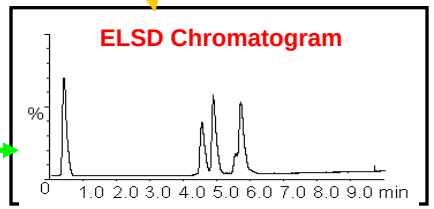






④ Normalized sample weights



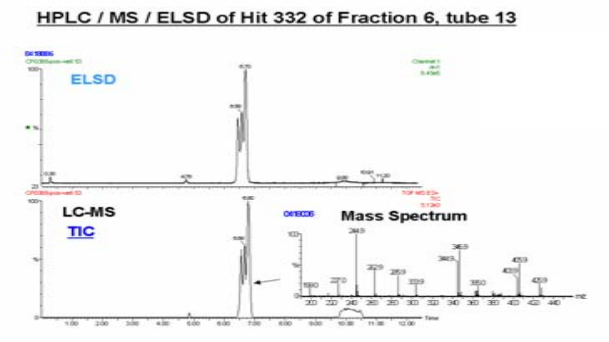


Utilize ELSD data to deliver normalized weights of each sample

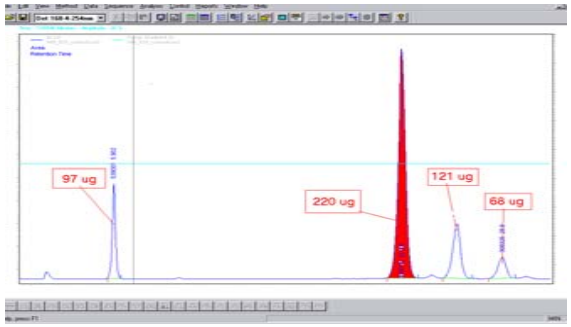
Screening concentrations between 1 to 20 µM

Biological Activity to Chemical Structure

1 **Collaborator identifies an Active Sample**



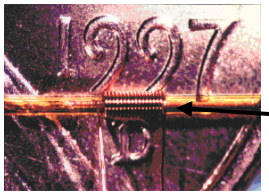
2 **Sequoia isolates compounds from an Active Sample**

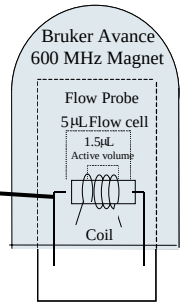


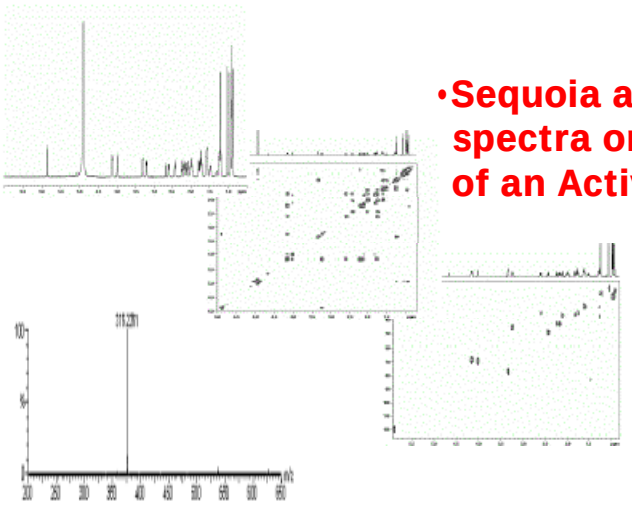
3 **Sequoia delivers compounds to Collaborator**

Collaborator rescreens purified compounds

4 **Structure elucidation of an Active Compound**

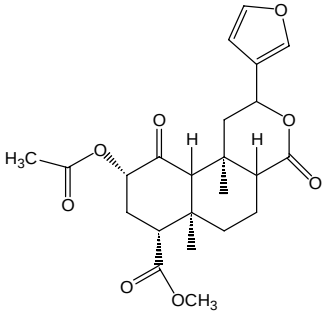






- **Sequoia acquires NMR spectra on 5 to 100 ug of an Active Compound**

- **Sequoia elucidates the chemical structure of an Active Compound**



What can I do with only micrograms of a sample?

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Move to Miniaturize


BRUKER
900
UltraStabilized
8. 7. 2001

Sequoia Sciences
High-throughput Natural Products Chemistry

CapNMR Probe

The Ultimate NMR Probe for Samples of Limited Mass

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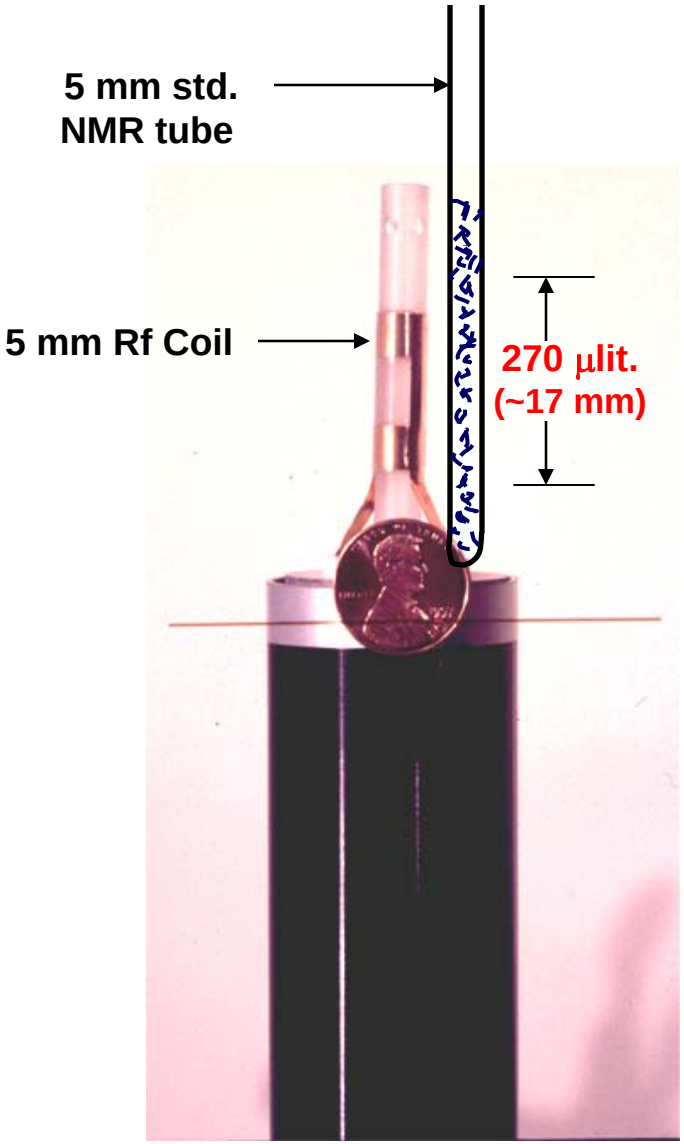
CapNMR™ Probe



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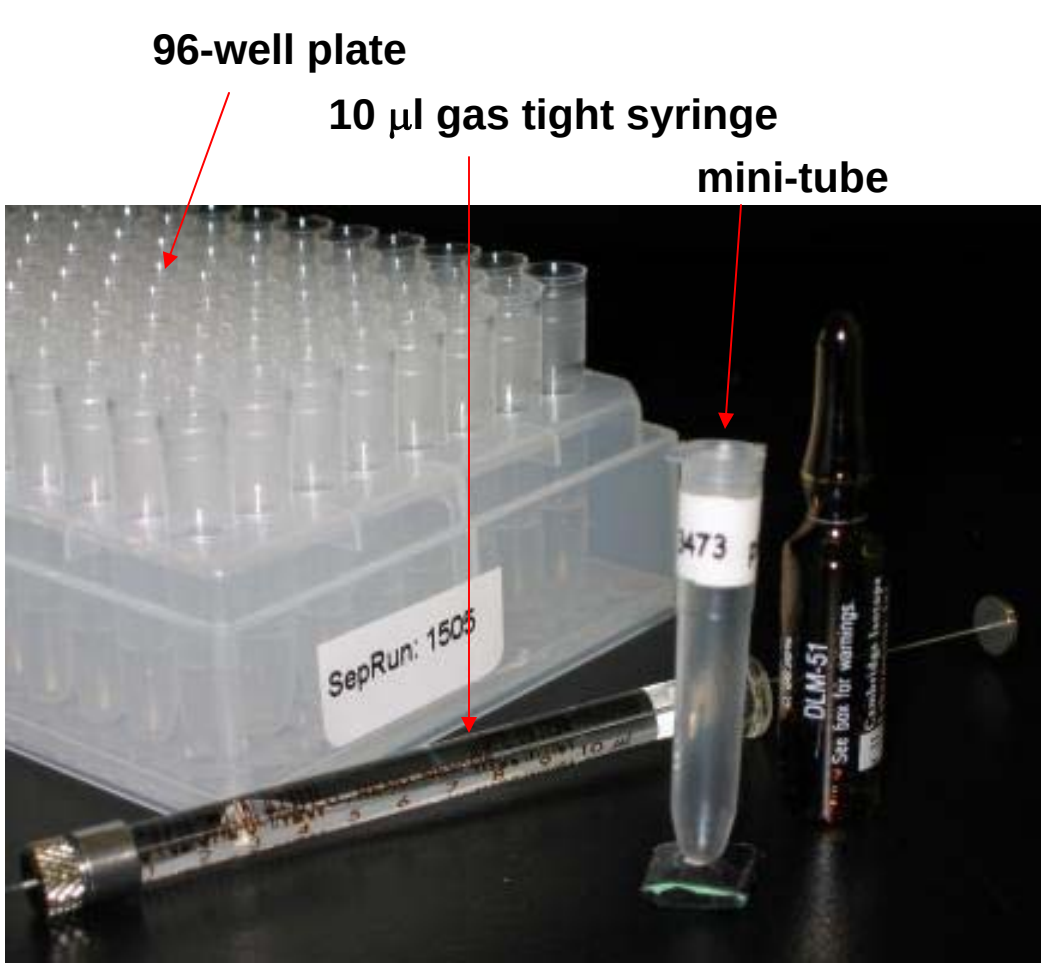
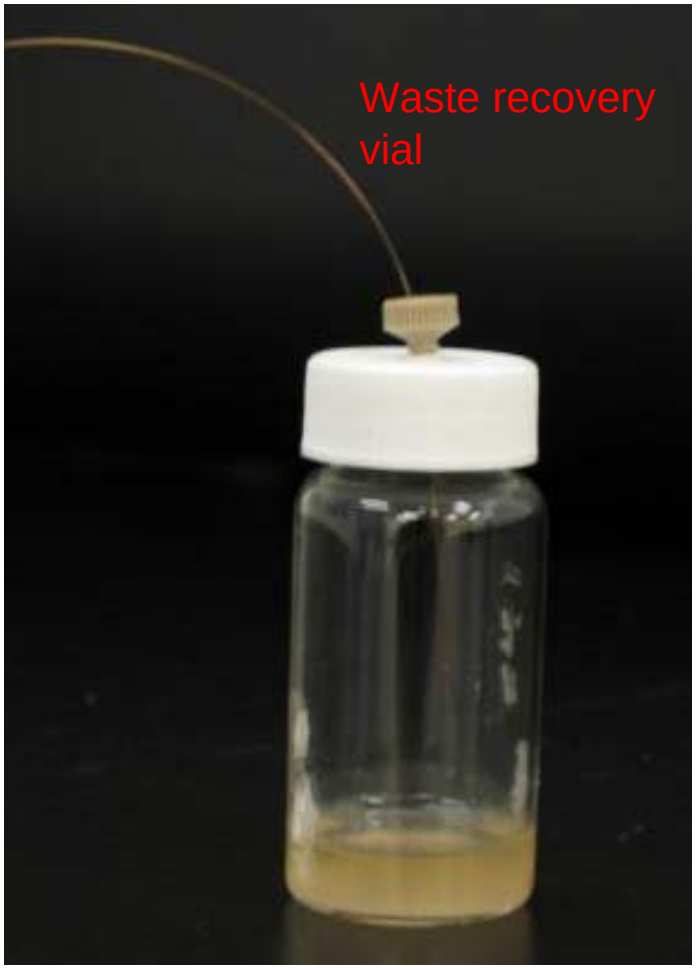
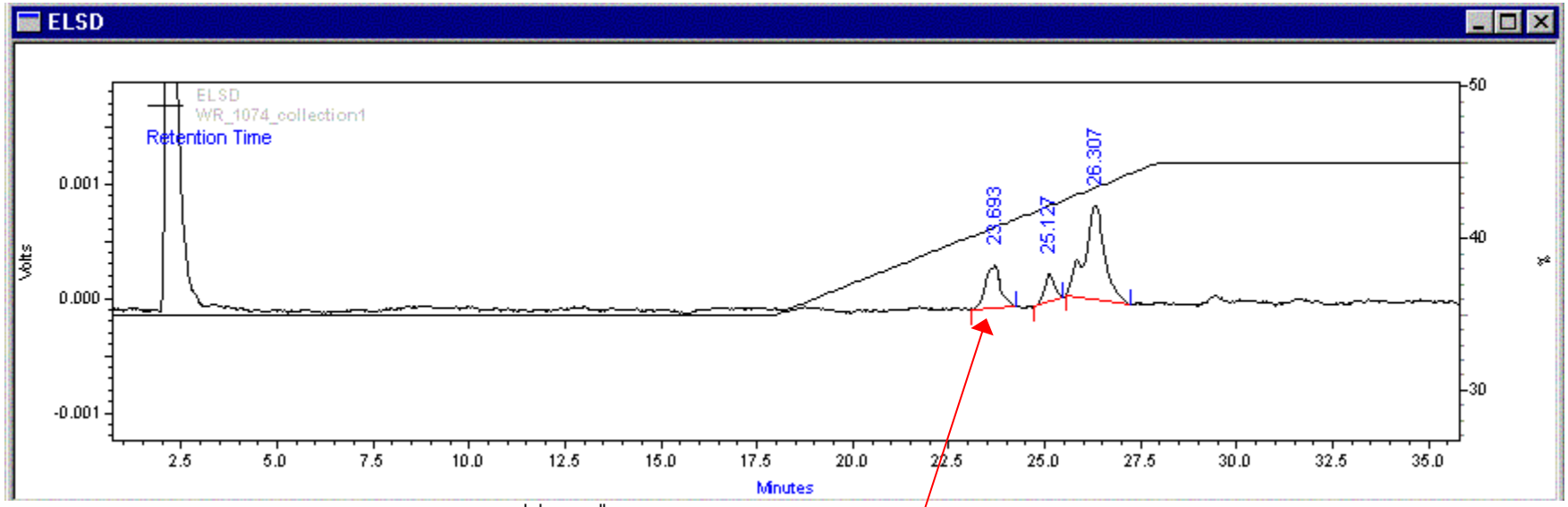
Sample Submission Hardware, Solvent and Waste Recovery



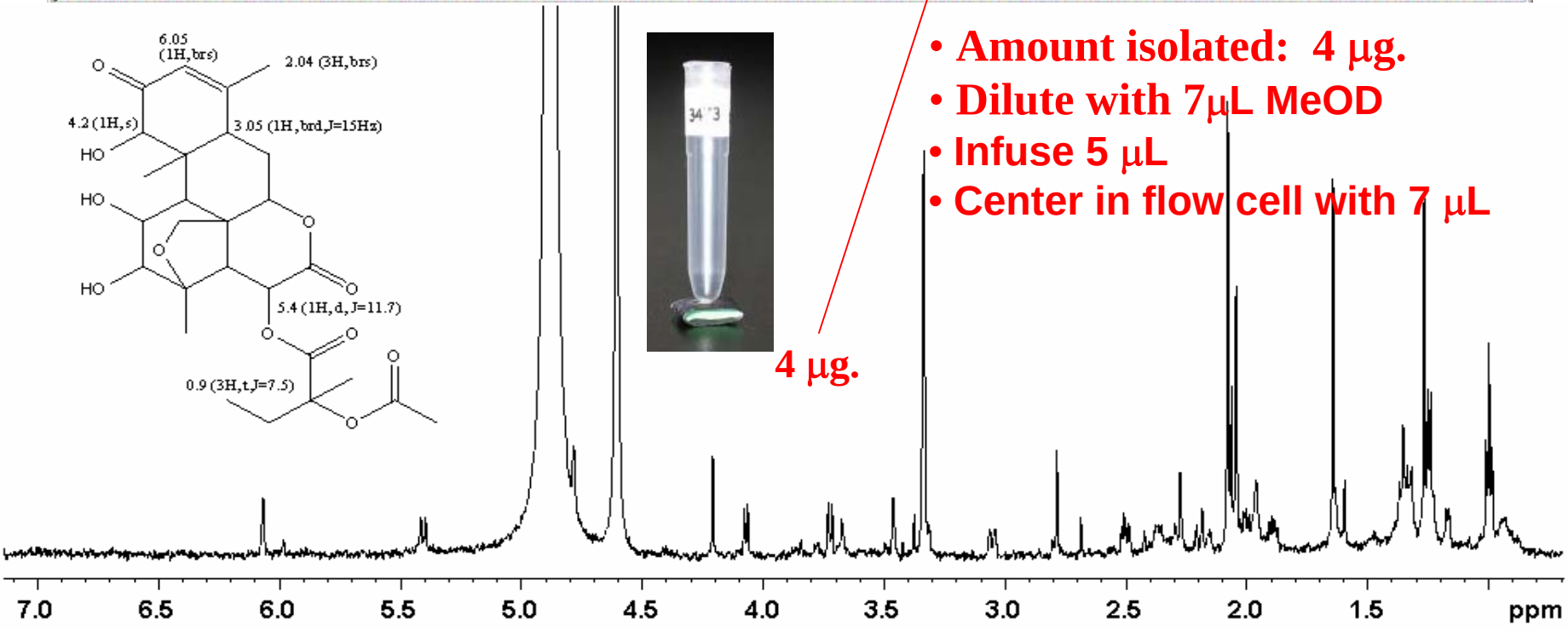


CapNMR™ Probe



4 μg.

- **Amount isolated: 4 μg.**
- **Dilute with 7μL MeOD**
- **Infuse 5 μL**
- **Center in flow cell with 7 μL**



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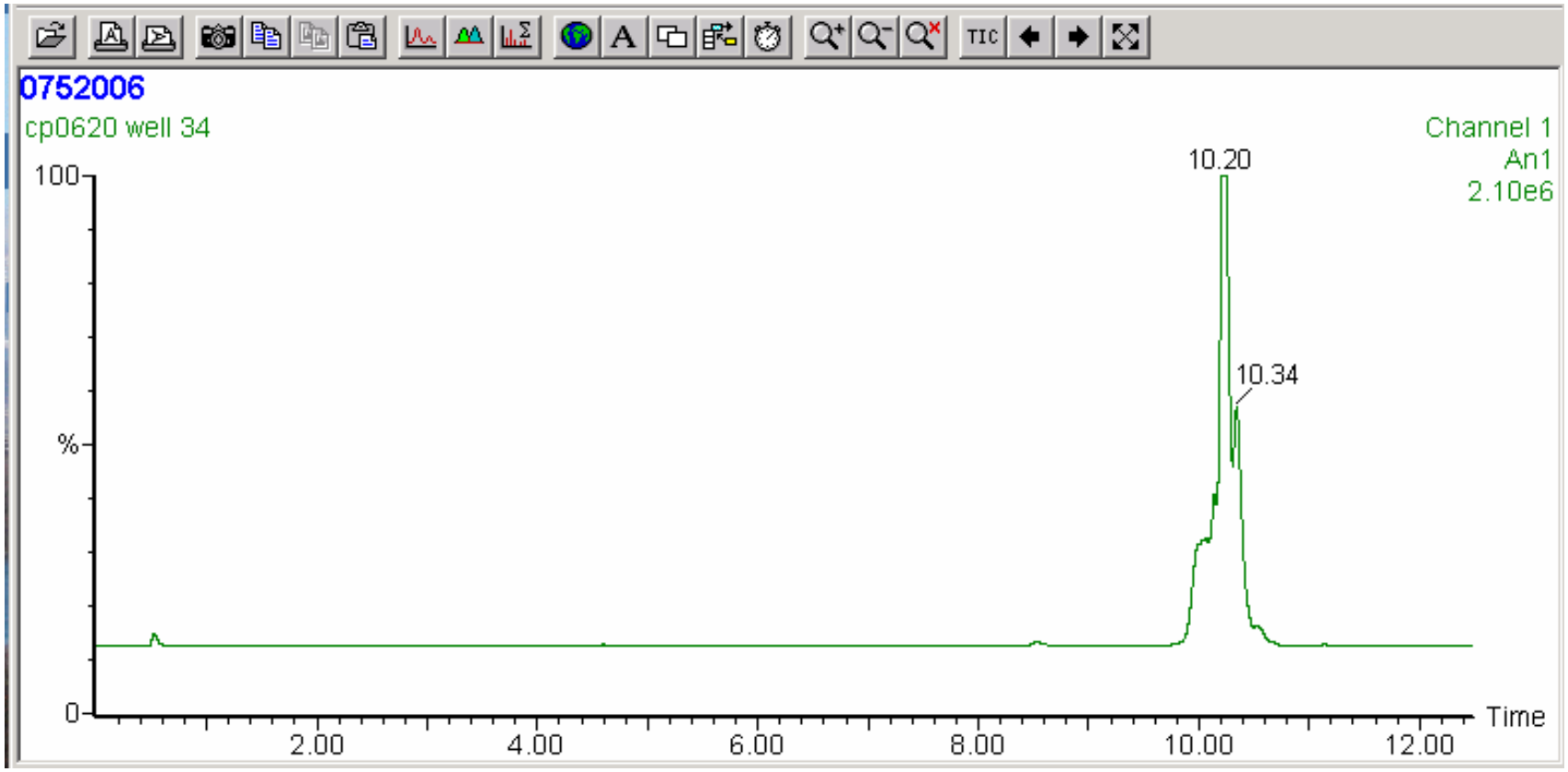
HPLC / ELSD of Hit 620 of Fraction 8, tube 34



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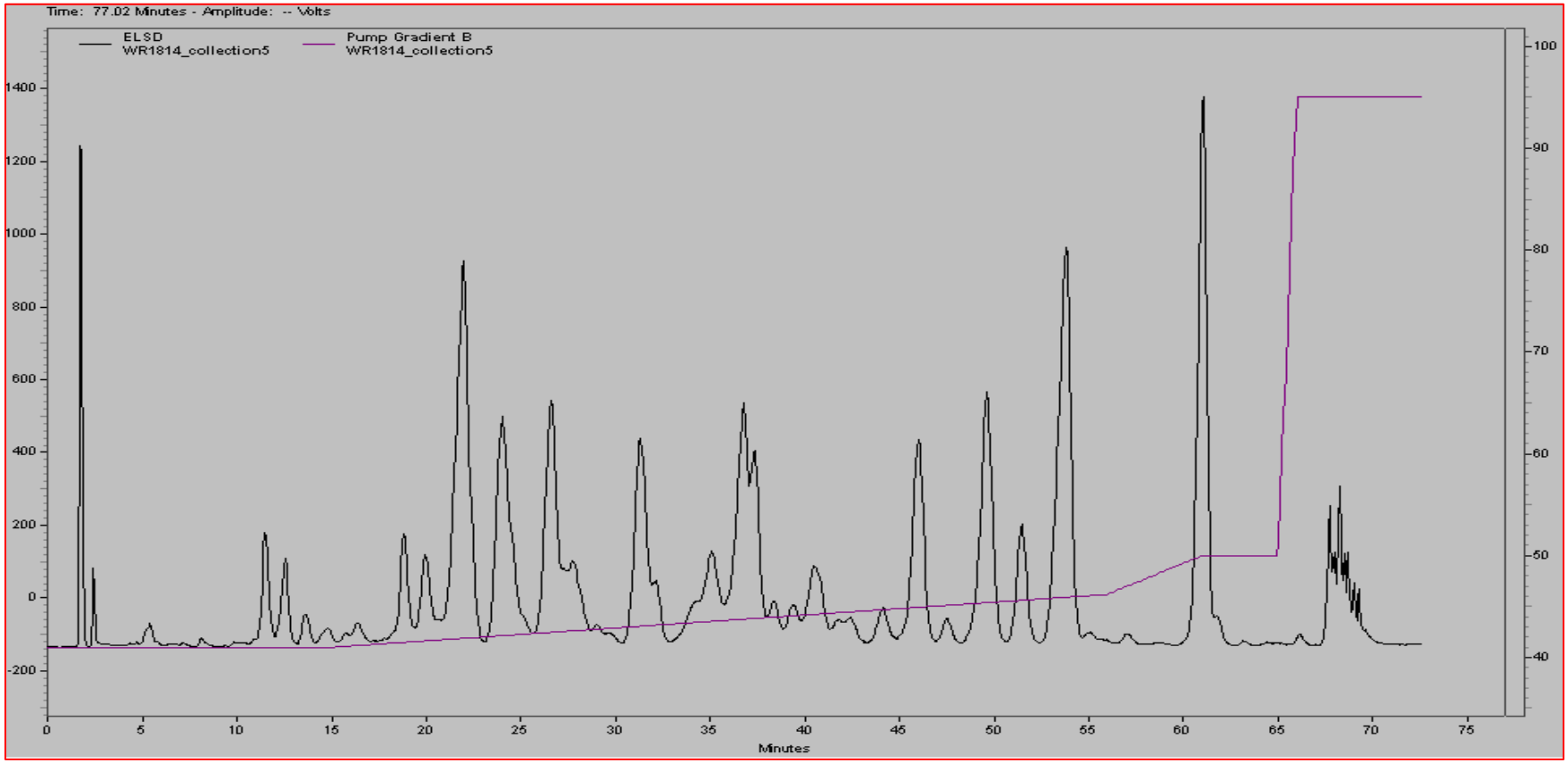
HPLC / ELSD of Hit 620 of Fraction 8, tube 34



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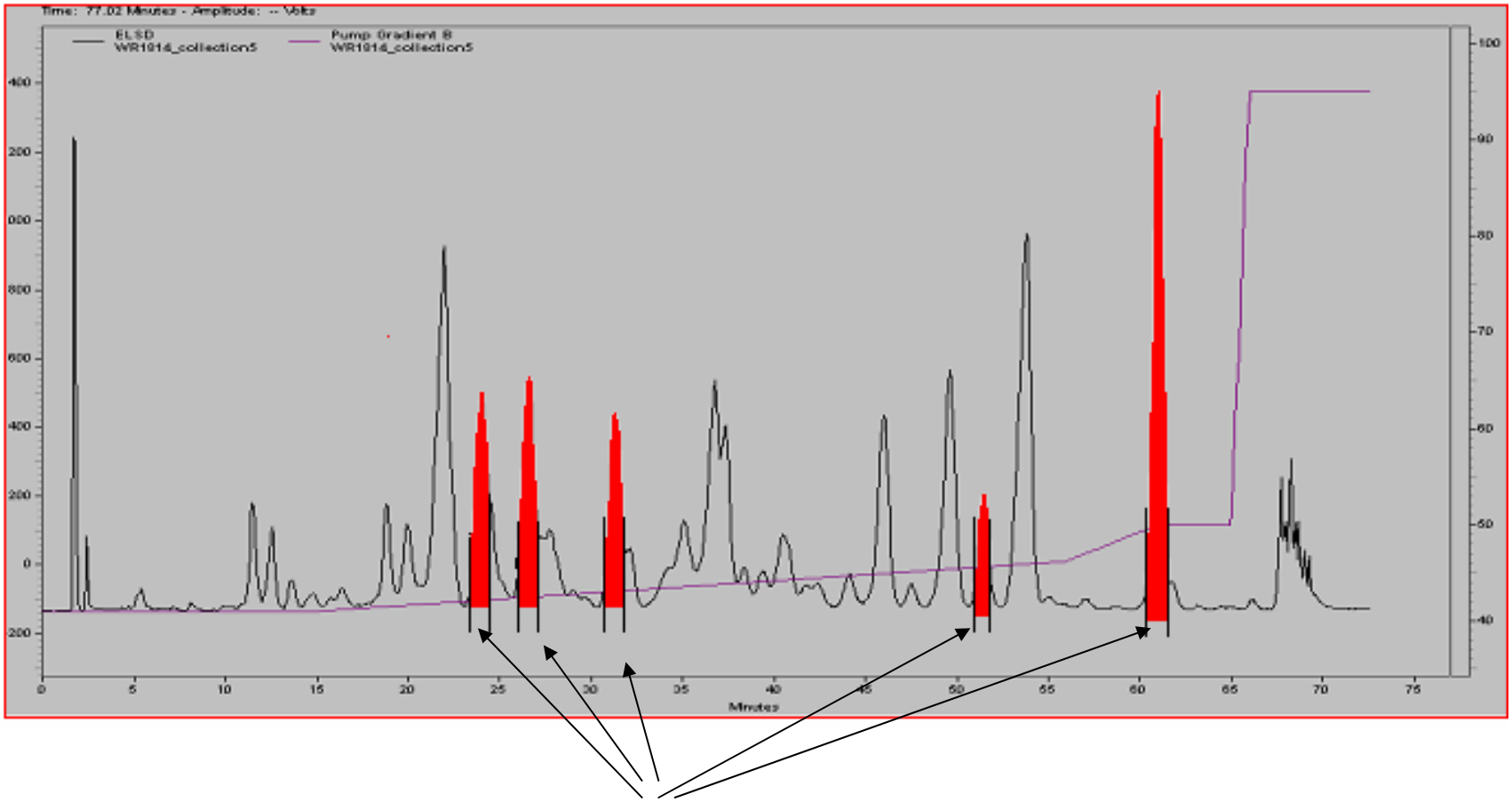
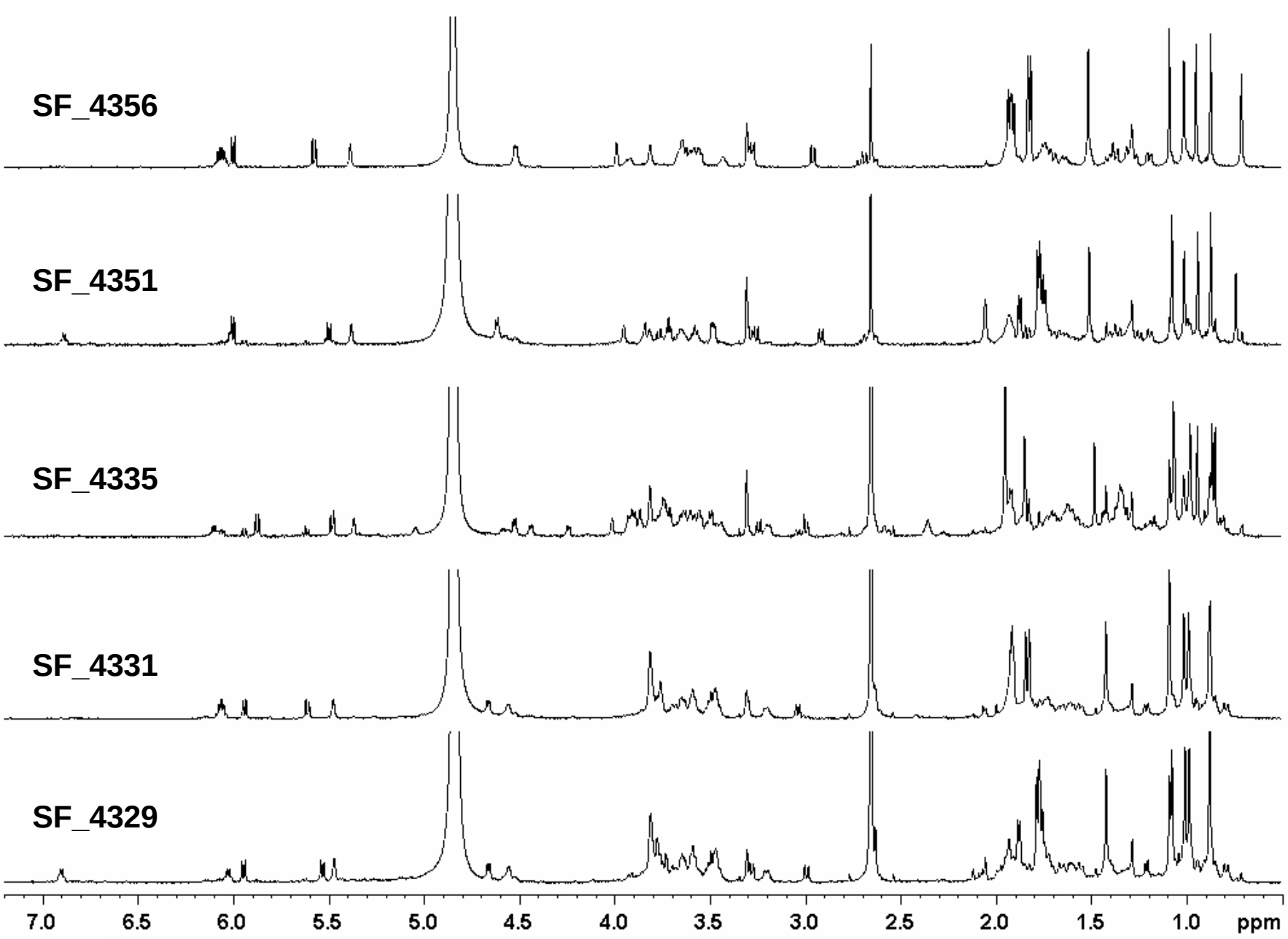
Active Fractions From Hit 620

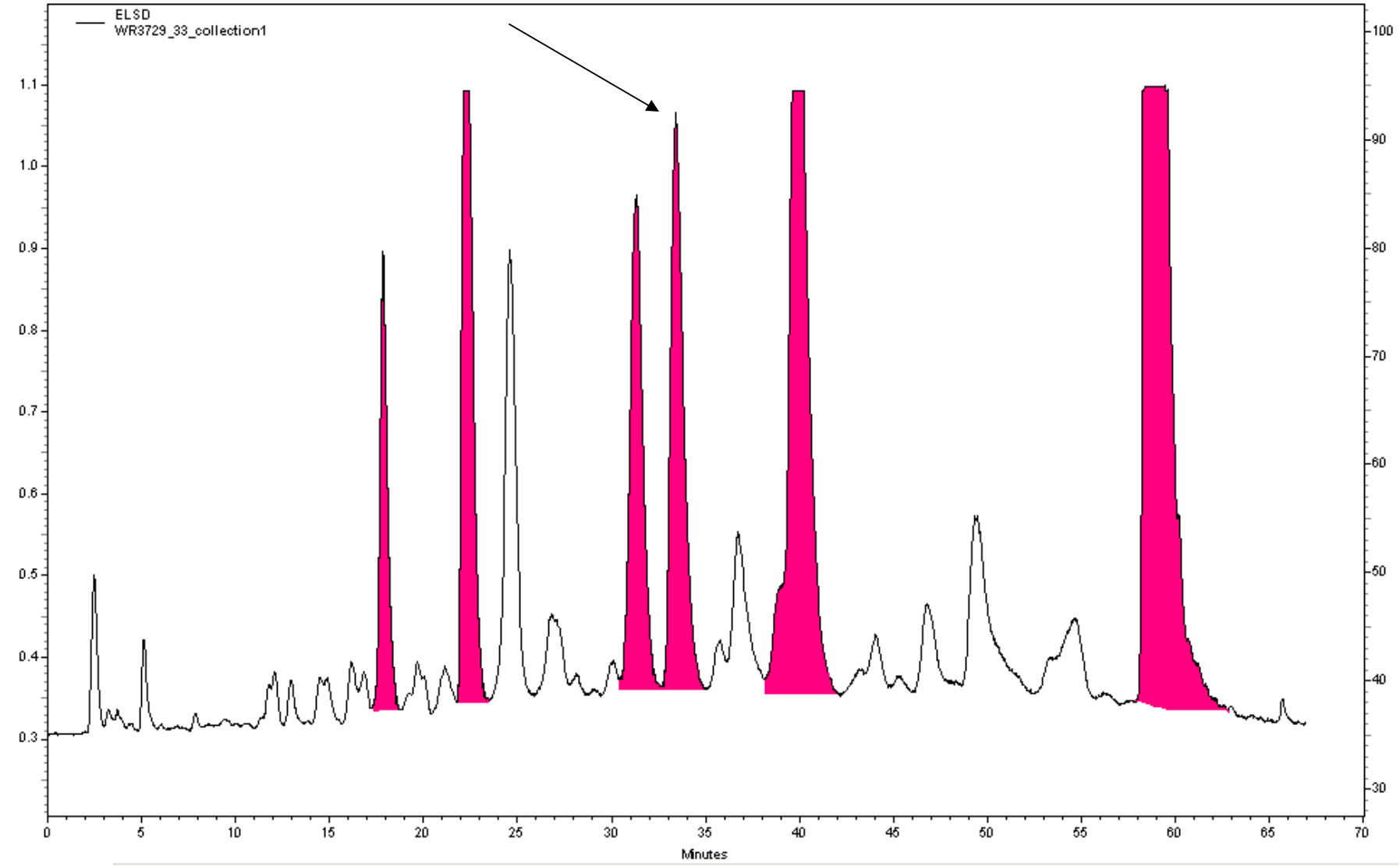


Active Fractions for Rescreen and NMR


SF_4356
SF_4351
SF_4335
SF_4331
SF_4329
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Plant Family: **Orchidaceae**

Species: ***Phragmipedium calurum***



High-throughput Natural Products Chemistry

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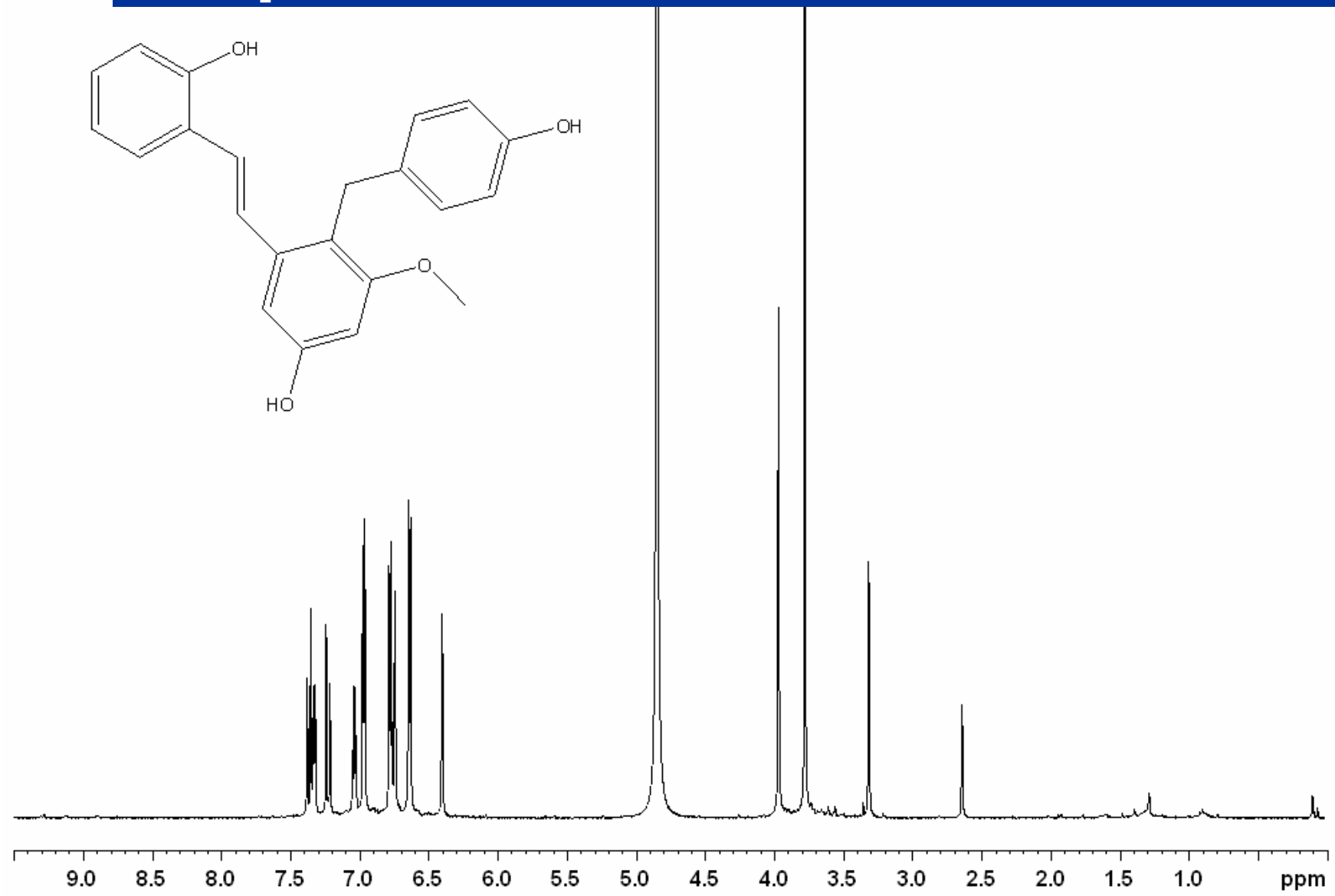
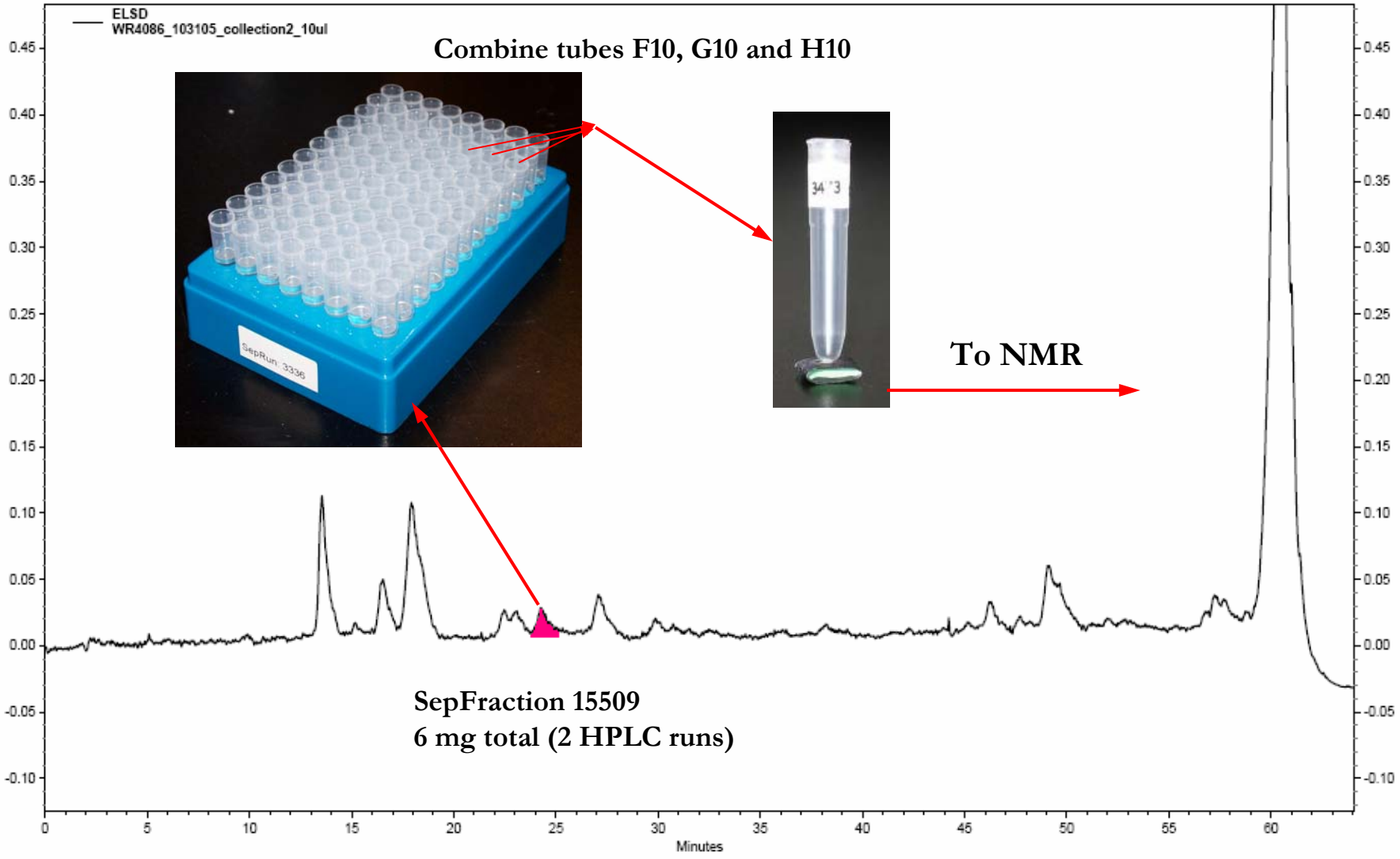


Plant Family: **Asteraceae**
Species: ***Layia platyglossa***



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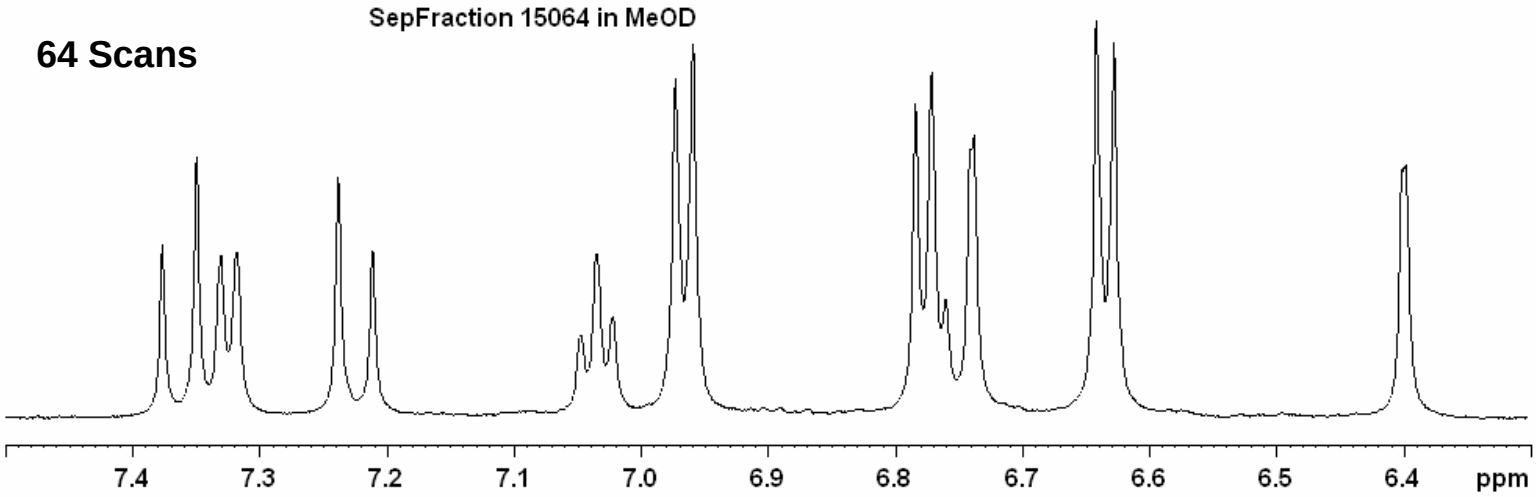
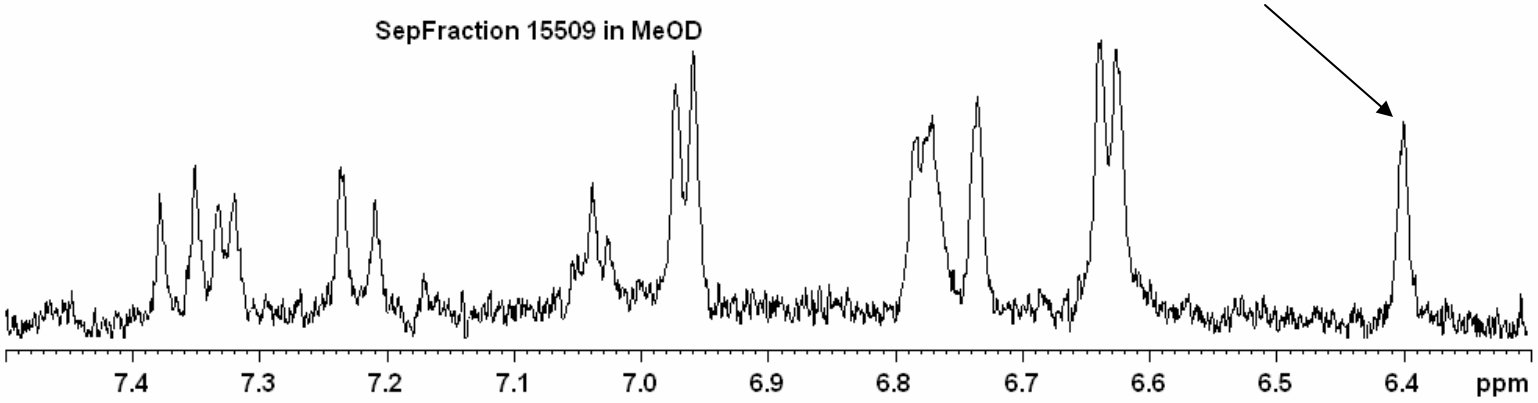
64 Scans



512 Scans

Data base peak search



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High-throughput Natural Products Chemistry

Bacterial Biofilms

Nasty Stuff!

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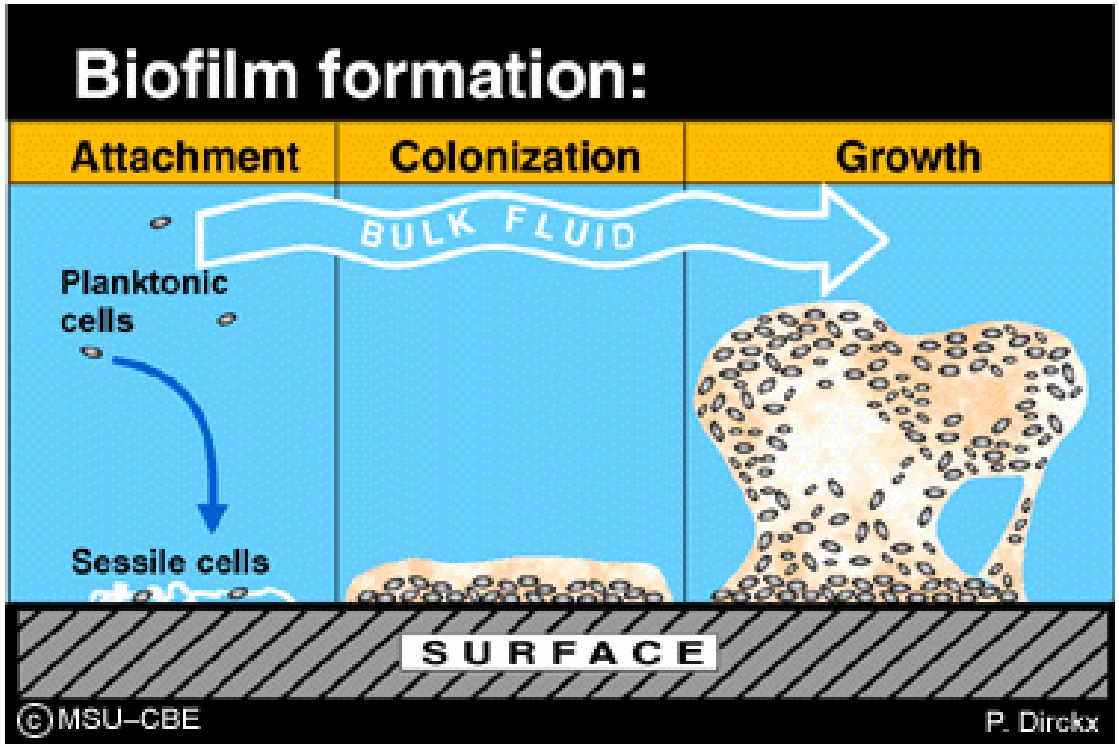
What is a Biofilm?

- Bacteria attach on surface, begin to secrete polysaccharide matrix
- Cells grow into a community, complete with nutrient channels
- Community thrives, protected by polysaccharide matrix



Type of Biofilms

Industrial Biofilms

- Ship bottoms and pipes
- Cooling towers
- Air ventilation systems
- Drinking water filtration and distribution systems
- Air filtration units
- Dental water lines
- Hemodialysis units
- Food processing systems

Medical Biofilms

- Urinary tract infections
- Lung infections
- Sinus infections
- Ear infections
- Chronic wounds
- Dental caries
- Medical device implants
- Acne

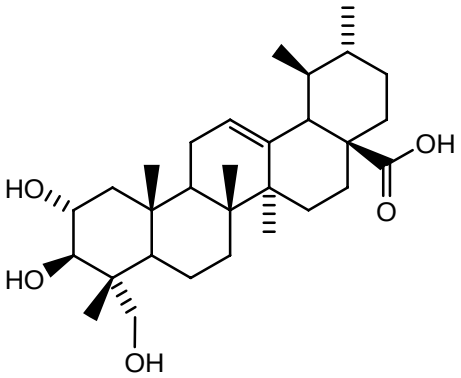
Biofilms

- Resistant to antibiotics at concentrations from 1000 to 1500 times higher than conventional use
- Involved in two-thirds of human bacterial infections
- No specific biofilm inhibitor is commercially available

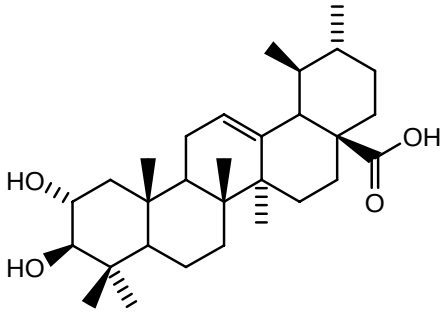
Screening for Biofilms

96 Well plate format for initial screen (initial screen at 10 μg/ml)

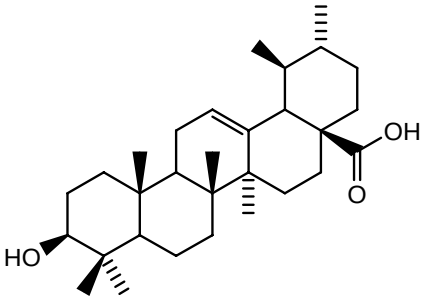
- **Asiatic Acid**
- **Corosolic Acid**
- **Ursolic Acid**



Asiatic Acid



Corosolic Acid



Ursolic Acid

Screening Challenge

- **Bacterial strain: *Pseudomonas aeruginosa* (PA01)**
- **Ciprofloxacin active against planktonic bacteria at 1 μg/ml**
- **Ciprofloxacin active against a bacterial biofilm at 50 mg/ml**
- **Develop a valid physiological screening assay**

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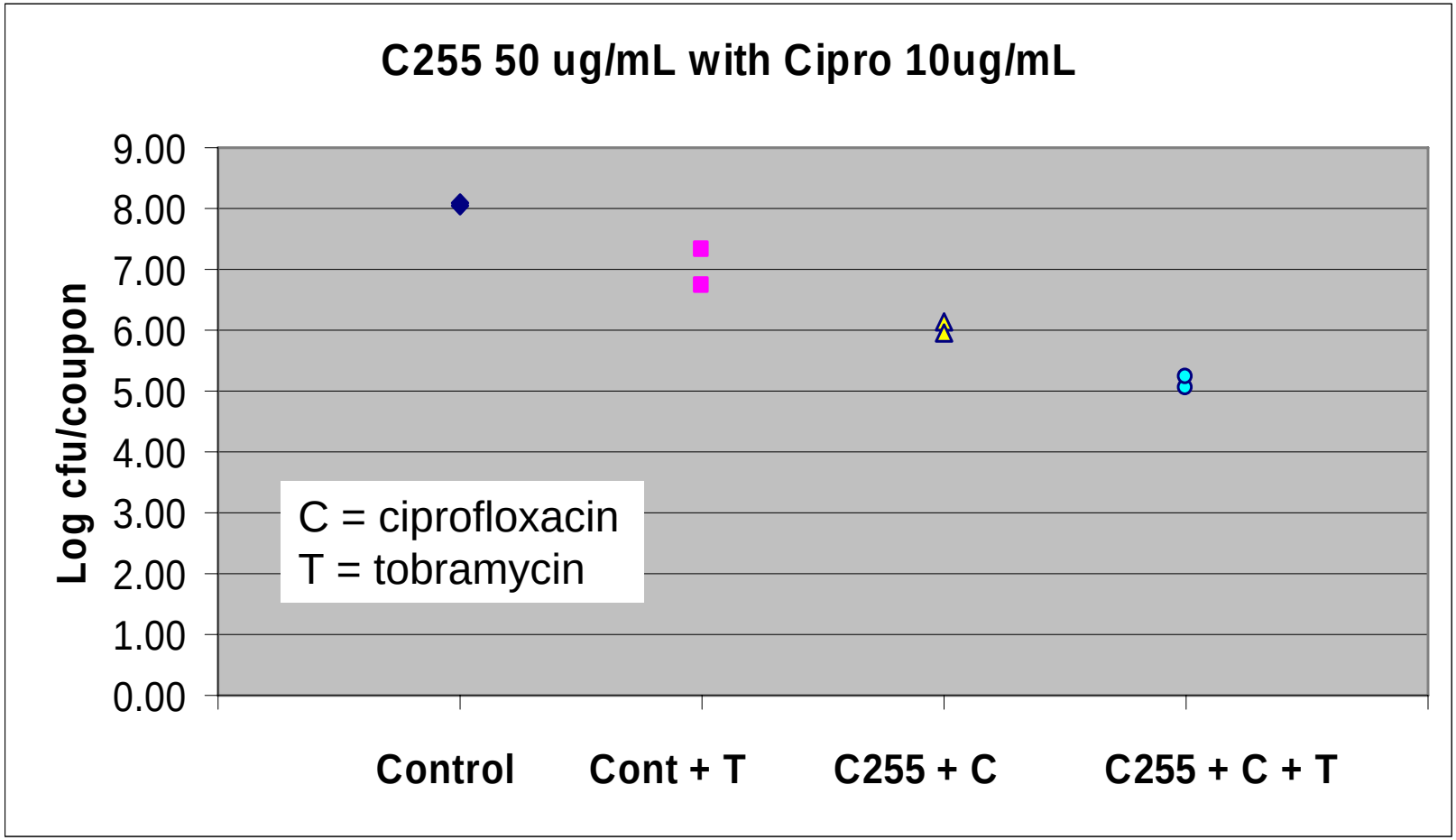
Rotating Disk Reactor (RDR)



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Tim Peck
Dean Olson

Bill Costerton
Garth James

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