

# LC-MS Purification in Drug Discovery

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Inform 2007, New Horizons of Laboratory  
Informatics Management. May 7-10, 2007, Miami, FL

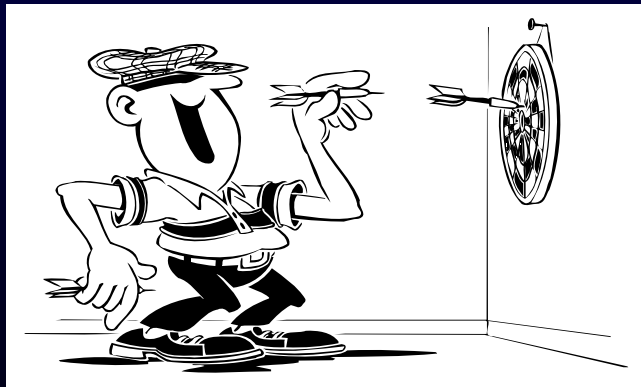
# Pathways of Drug Discovery



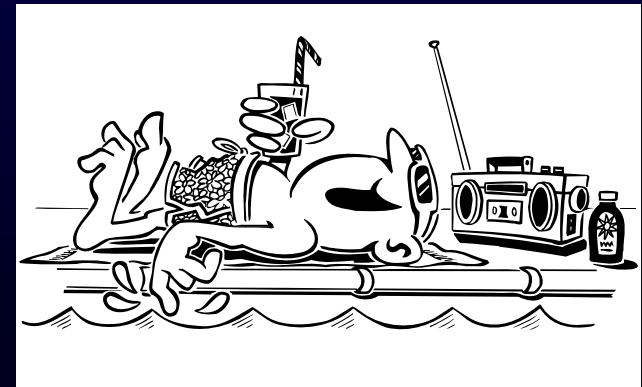
Target & hit  
Identification



Hit refinement & Lead optimization



Selected Clinical Candidate

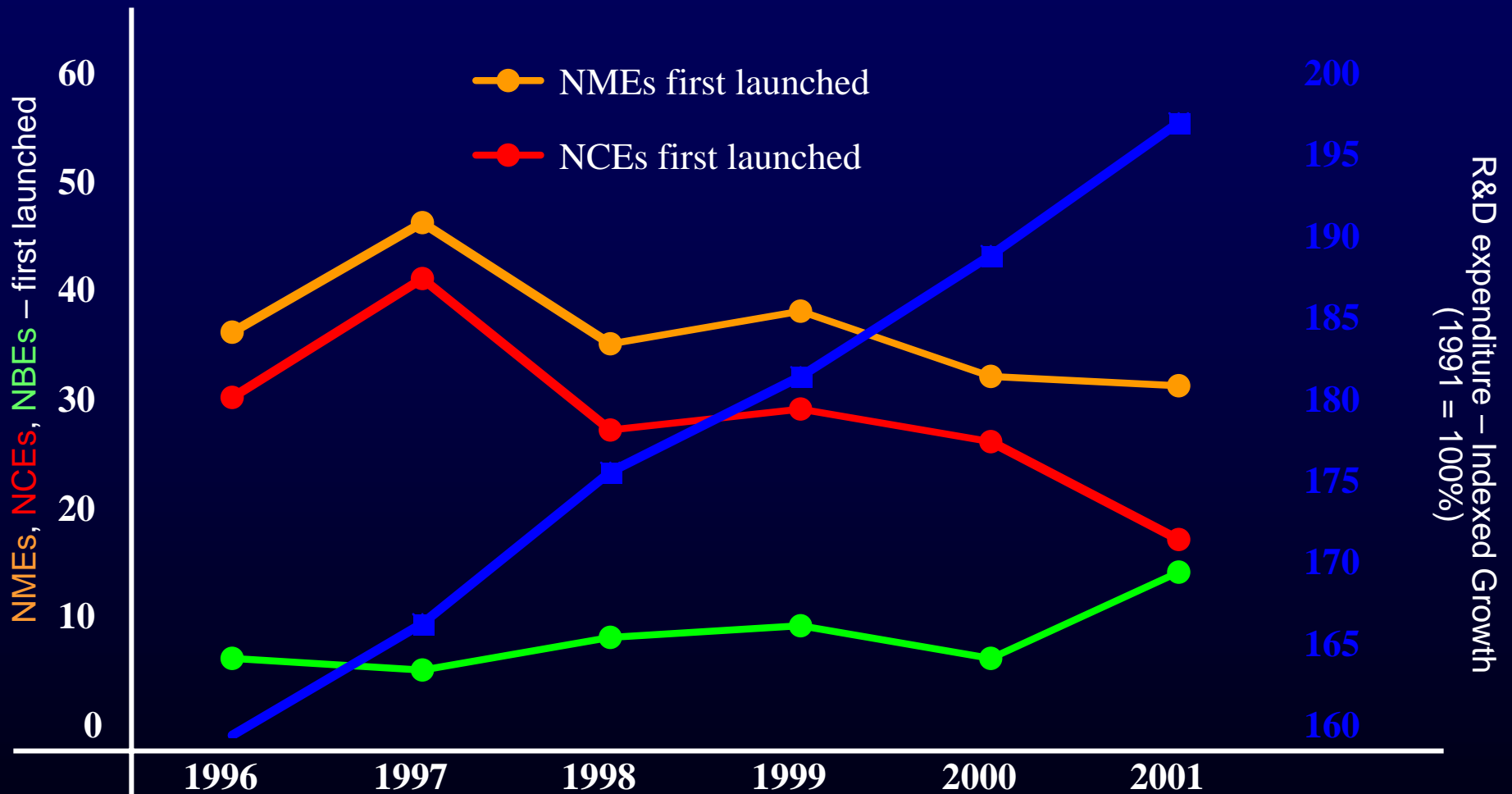


Marketed Drug

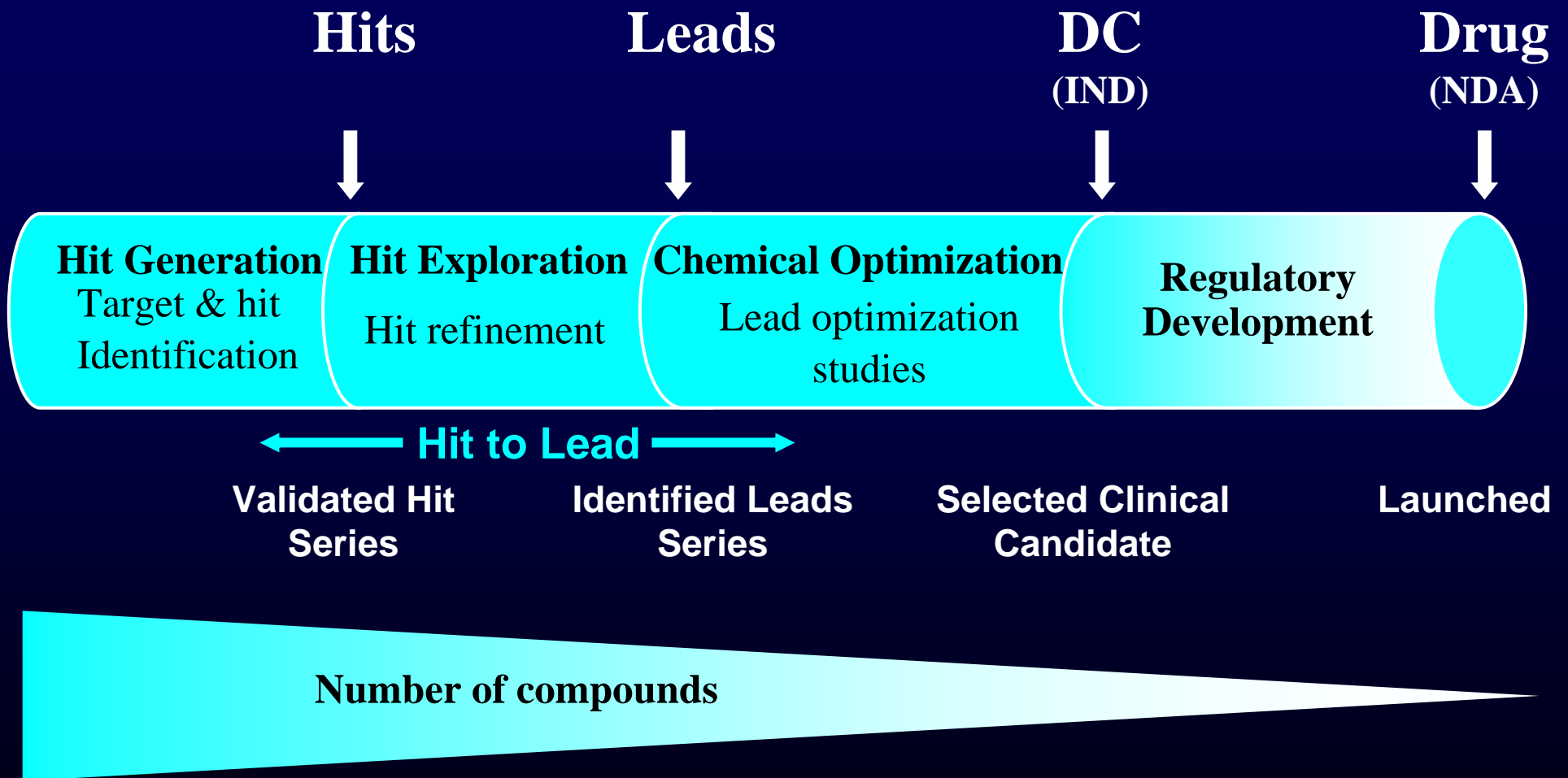
- **Introduction**
- **Lead Generation Libraries**
- **Purification Group Core Activities**
  - **Open Access LC-MS**
  - **Dedicated Support Group**

# Global R&D Productivity Trend (1996-2001)

Increasing costs while stagnating output



# Decision Gates in Pre-Clinical Drug Discovery

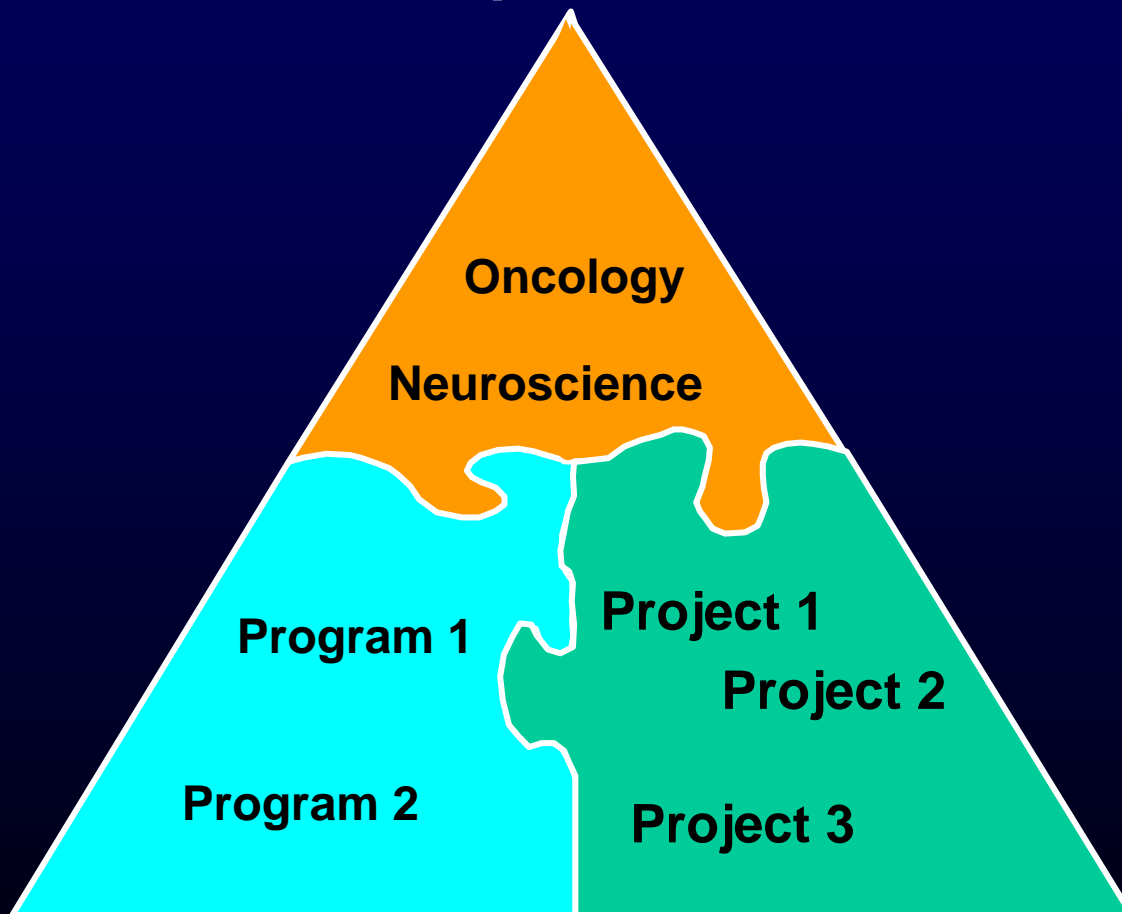


**Stage-by-stage quality assessment. Reduce attrition rate, time and cost.**

- **Introduction**
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# Objectives of Hit to Lead

Compound Collection  
Expansion



Support  
Medicinal  
Chemistry  
Programs

Progress  
Hits to  
Novel Leads

# What is a Lead Structure ?

- Desirable biological activity
- SAR showing modulation of biological activity as well as other parameters
- Not an extremely polar or lipophilic compound.
- No toxic group or groups that will produce toxic metabolites
- Not irreversibly reacts with its biological target
- Molecular weight and lipophilicity range
- Rule of 5
- Polar surface area
- Chemical feasibility
- Good probability to deliver a clinical candidate.

**Lead structure optimization is an evolutionary procedure.**

“Chance only favors the prepared mind”, Louis Pasteur

# Lead Generation Libraries Workflow



Target Families  
Ligands  
2D/3D  
Pharmacophores  
Homology Models  
X-Ray  
Structures

Scaffolds  
Reagents  
Building  
Blocks  
Acquisition

Solution-/  
Solid-  
Phase  
Solid-phase  
Supported  
Reagents  
Microwaves

RP-LC  
SFC  
SiO<sub>2</sub>  
Extraction  
Crystallisation  
Absorption

LC-MS  
NMR  
IR  
UV/ELSD

Data  
Merging  
Archiving  
Registration

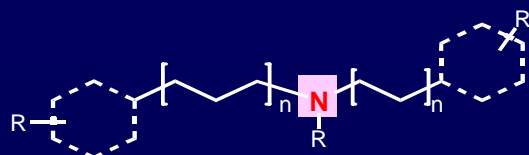
Biological  
Screening

Knowledge-based Design  
High Quality Data  
Automation  
Data Integration & Data Sharing



**Better Compounds  
Faster**

# General Structures of Libraries Members

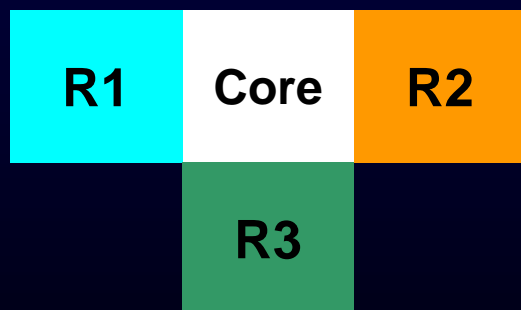


Hydrophobic – Basic -Hydrophobic

Design Principle



Library design

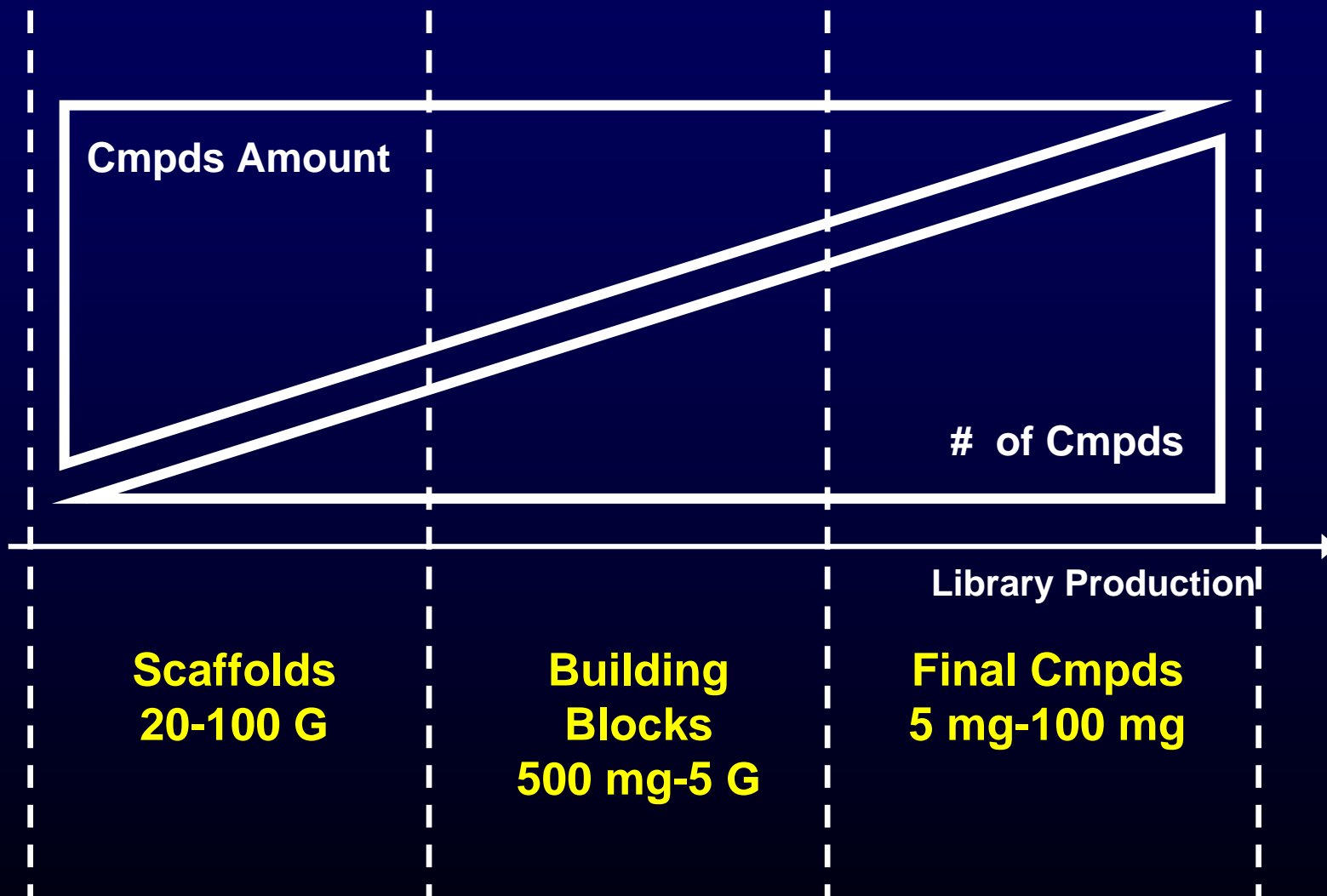


Final Compound

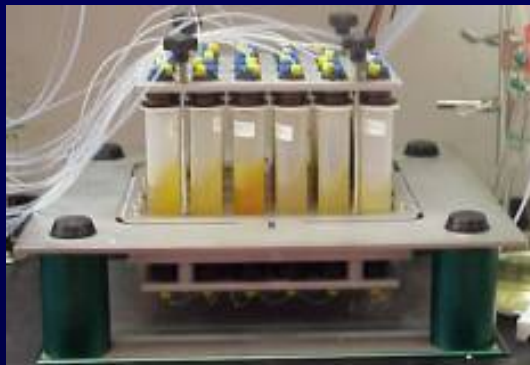
Bearing hydrophobic, Hydrophilic, basic, Acidic, neutral groups

Library Synthesis

# Overview of Library Production



# Integrated Synthesis Technologies



CCS24 Synthesis Station



Syncore (4x24, 4x96)



Microwave



Four-Season Shaker

- **Introduction**
- **Lead Generation Libraries**
- **Purification Group Core Activities**
  - **Open Access LC-MS**
  - **Dedicated Support Group**

# Purification Group

## Objectives

- Support group (Libraries & Medchem cmpds)
- Optimal use of high-value equipment

- Provide full service & open access mode.
- Deliver both quality and speed to the customers

## Group Core Activities

## Challenges

- Number of samples
- Productivity: Purity > 90%  
Recoveries >75%  
Success rate 100%  
Throughput  
Turnaround time

- Yield range
- Amount range
- Structural complexity
- Synthesis
- Structures:
  - Lability
  - Solubility
  - Chromophore
  - Isomers

# Integrated Purification Technologies



# LC-MS in Open-Access Mode

## Analytical LC-MS

- Quick (< 2min) information-rich analysis.
- Spontaneous & time-flexible use.
- Reaction monitoring, routine analysis, purity assessment but not for method development.
- Replacement for TLC.

## Preparative LC-MS

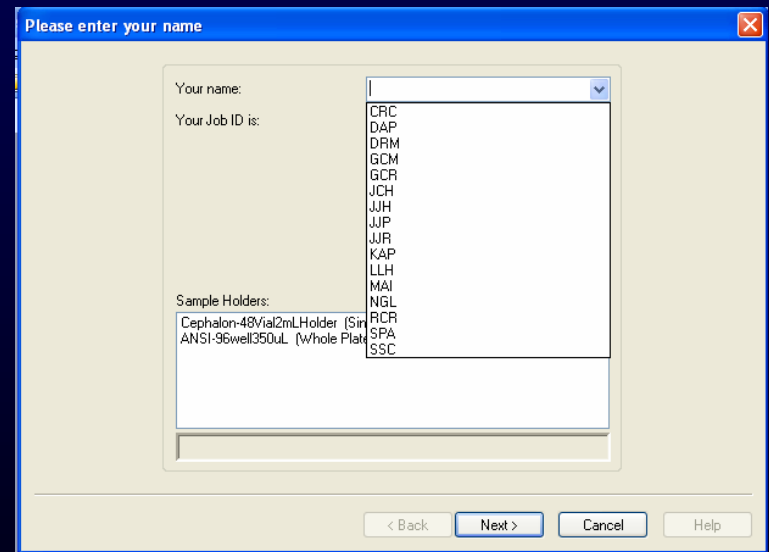
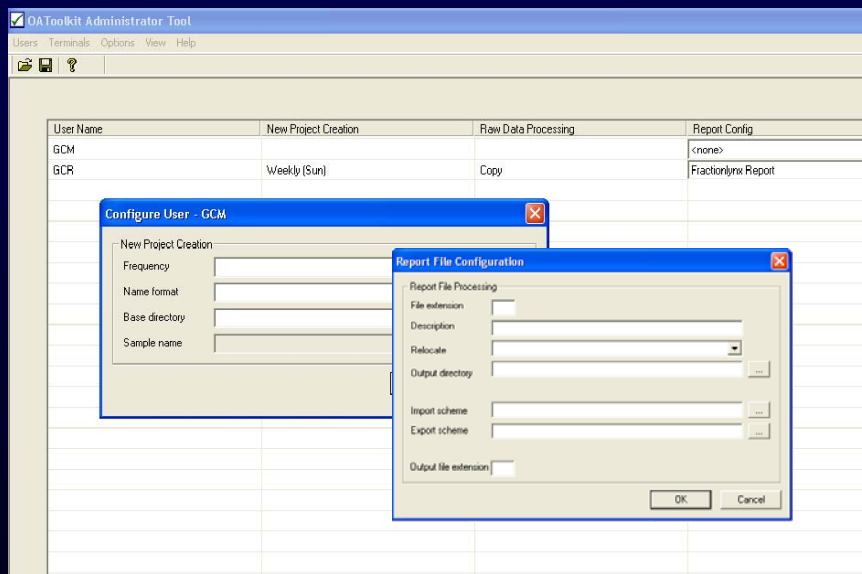
- Rapid purification (< 10 min).
- Spontaneous & time-flexible use.
- Intermediates, final products.

## Challenges

- Instrument maintenance.
- Users Training.
- users skills level & discipline.
- Data archiving & sharing.

# OALogin ToolKit Service

- Creation and management of users list(s).
- Password protection.
- Enabling relocation of raw datafiles, report files, and batch files.
- Converting report files to different formats (XML, HTML, .txt).



# Integrated Data Management System

Acquity LC-MS PC



Fractionlynx PC



Universal Workstation PC



Data Storage PC



Users PC  
Data Report File

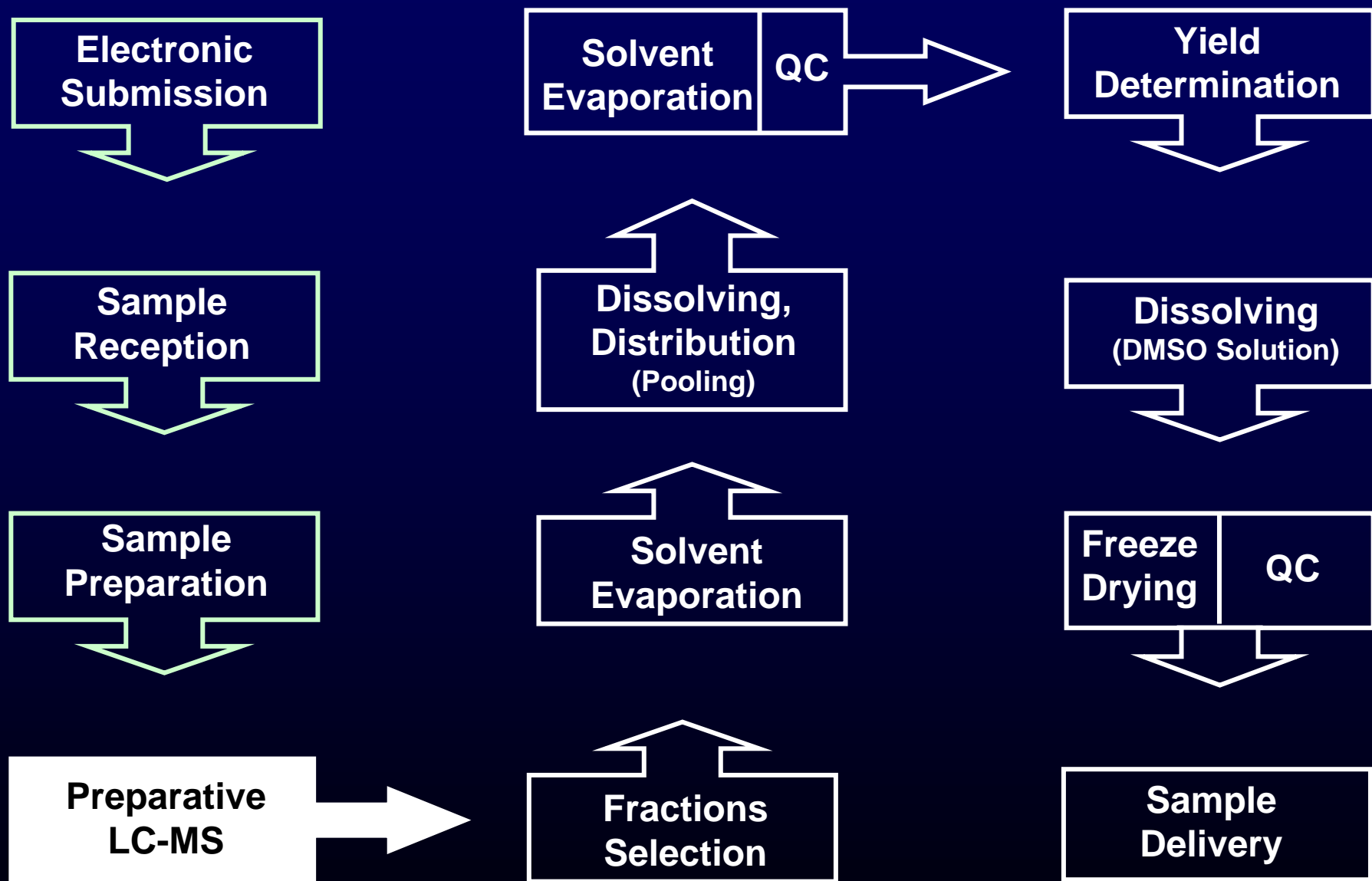


Label/Barcode  
Printer

Corporate Network

- **Introduction**
- **Lead Generation Libraries**
- **Purification Group Core Activities**
  - **Open Access LC-MS**
  - **Dedicated Support Group**

# Samples Purification Workflow



# Pre-Preparative Chromatography

Electronic  
Submission



- Email submission via Cephalon Intranet
- Completed Sample File
- Link/Copy of o/p./f/p report. Analysis on Acquity LC-MS

Sample  
Reception



- 96-Well Plate Format
- Dedicated location
- Shipment from different sites

Sample  
Preparation



- Dissolving in DMF
- Filtration
- *“Quick” clean-up (Silica Gel)*

Preparative  
LC-MS



# Electronic Samples Submission

Electronic  
Submission

Flp report can be viewed from Users PC.

<https://intranet.cephalon.com/dr/doctlist.asp?cat=578> - Microsoft Internet Explorer provided by Cephalon, Inc.

**Cephalon** deliver more > Innovation

Hit To Lead - Purification Group

Name: **Purification Group**

Description:

**General Information**

- Samples should be submitted in a 96-deepwell plate format unless there are fewer than 10 samples. If in solution, sample should be less than 1 mL.
- Purified samples will be returned in 1 DRAM vials. A product file containing the sample amount and % purity at 220 nm will together with the LC-MS report file.
- Progress of the purification of your samples can be followed by viewing the [purification\\_queue\\_file](#)

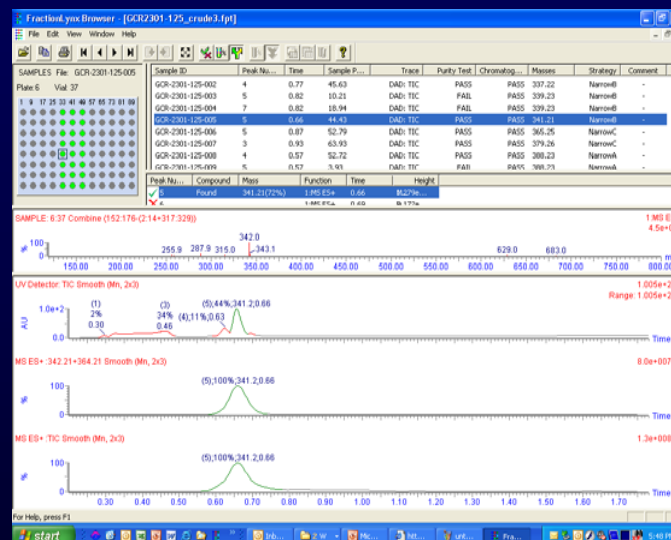
**Sample Submission**

**Step 1:** Open [ProductsCEP\\_template](#) and fill columns A (CHEMISTRY), B (Notebook\_Code) and E (Well ID).

**Step 2:** [Email](#) your completed Accord file to the Purification\_Group along with the information listed in the email.

**Step 3:** Bring your samples to the purification group members or place your samples in box labeled "Purification Group" in the ref lab S114.

Last Update: 9/14/2006  
Format: Microsoft Word Document



Analytical data acquired using  
Acquity LC-MS (flp reports).

# Analytical LC-MS

## Hardware & Software

- Acquity LC-MS (ZQ & SQD).
- Diode Array & ELSD.
- Single vials and/or plates.
- Masslynx 4.1.
- Running Fractionlynx methods via Openlynx Login.
- 

## Column Chemistries & Methods

- BEH C<sub>18</sub> 1.7 microns 2.1 x 50 mm column.
- Flow: 0.6 mL/min.
- “Generic” methods.
- ESCI pos. or neg. mode.
- Chemical reaction monitoring.
- Final purity assessment (QC).

# Preparative Chromatography

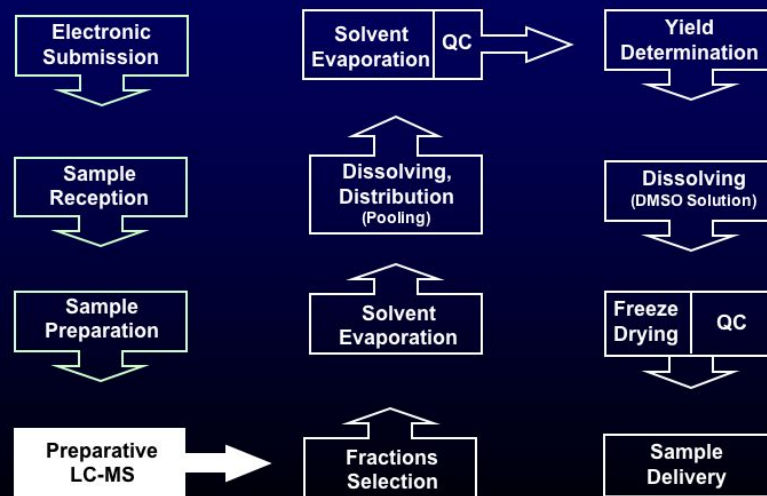
## Challenges

- Number of samples
- Yield range
- Amount range

**Productivity:**  
Purity > 90%  
Recoveries >75%  
Success rate  
100%  
Throughput  
Speed

**Structural complexity**  
**Synthesis**  
**Structures:**  
**Lability**  
Solubility  
Chromophore  
Isomers

## Samples Purification Workflow



# Preparative LC-MS

## Hardware & Software

- Fractionlynx System.
- Masslynx 4.1.
- Custom made collection racks & collection tubes.
- At column dilution for both analytical & preparative LC-MS.

## Column Chemistries & Methods

- Xbridge OBD C18, 5 microns (19 x 50 mm).
- Flow: 35 mL/min.
- Sample specific gradient, selection using Autopurify.
- Mass-triggered collection.
- Crudes samples (4-120 mg) dissolved in DMF.
- Injection volume < 1 mL.

# Preparative Chromatography - Hardware

## Fractionlynx System



**CFO:**  
2 preparative columns  
1 analytical column

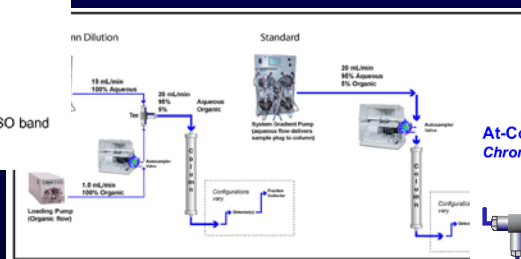
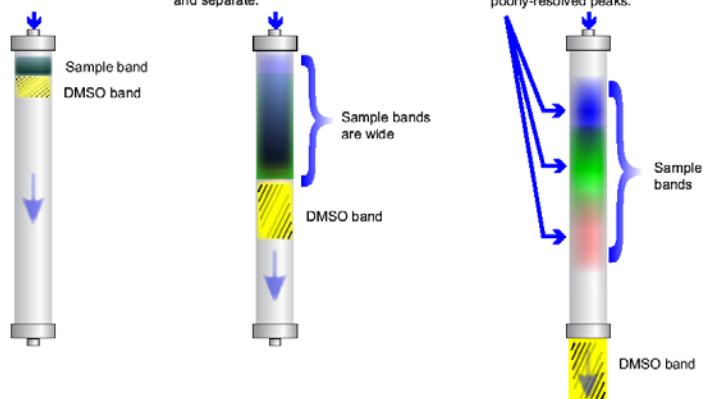
**Custom made racks  
and fraction tubes**

**“Modifiers”  
515 pump**

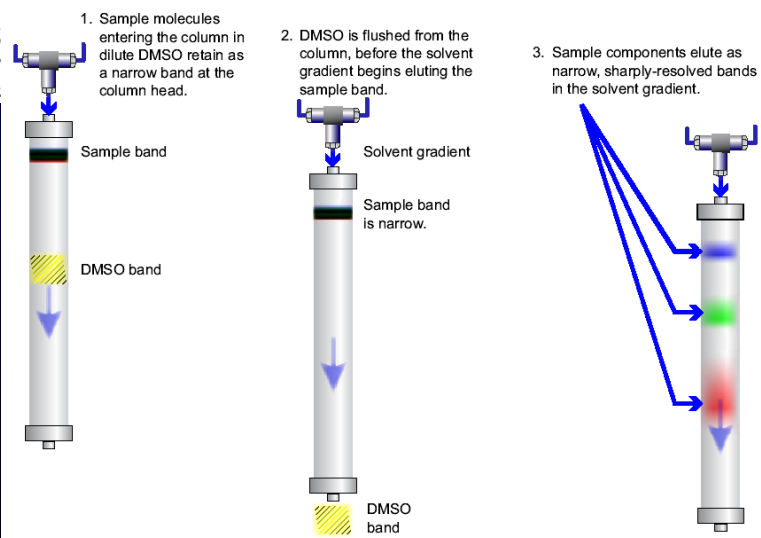
# At-Column-Dilution Separations

## Conventional Separations Chromatography Diagram

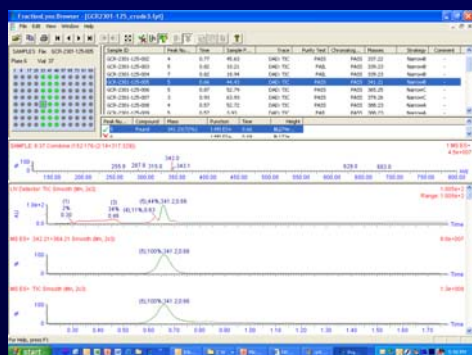
1. Sample molecules entering the column in the DMSO plug do not retain.
2. The sample and DMSO bands dilute with initial-strength mobile phase as they move through the column. As the DMSO dilutes, the sample components begin to retain and separate.
3. Sample components retain on the column as wide bands. They elute during the gradient as broad, poorly-resolved peaks.



## At-Column-Dilution Separations Chromatography Diagram



# Autopurify - Gradient Selection



Report from analytical  
LC-MS

MassLynx - Hit2lead - GCR-2301-125repeatP7.SPL

File View Run Help

Shortcut Queue Status

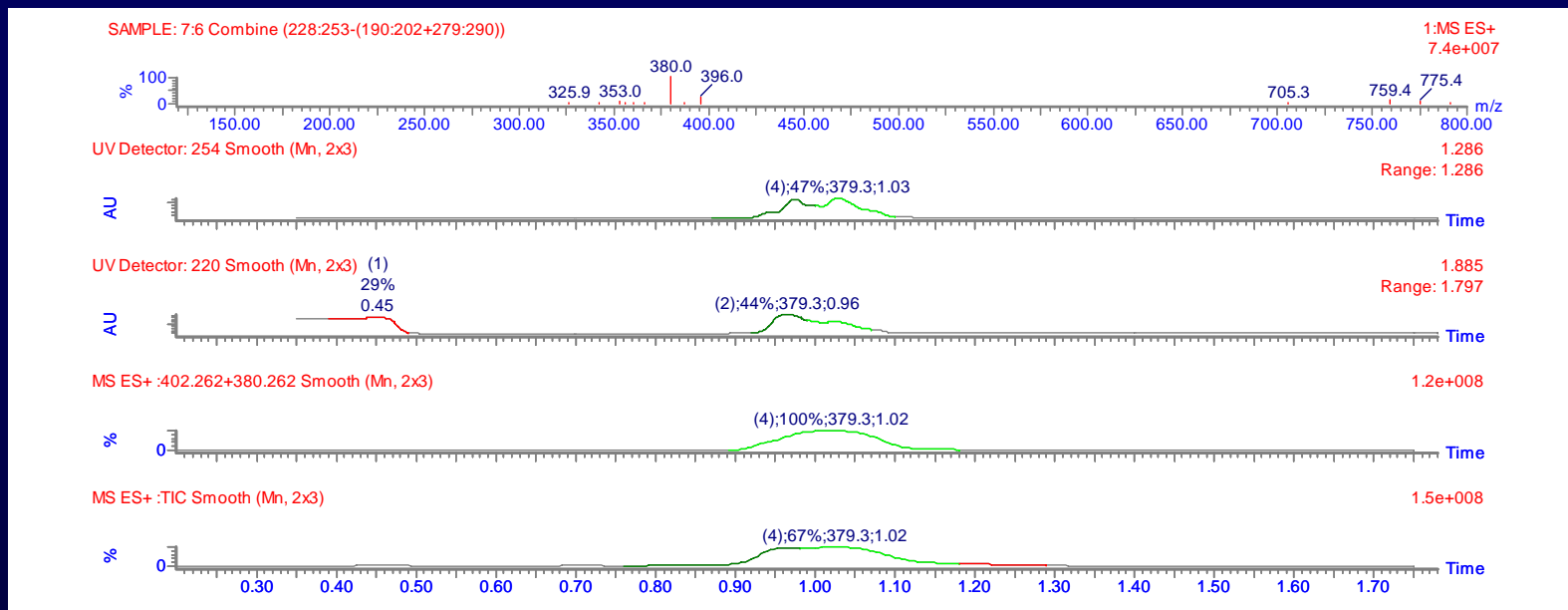
GCR-2301-125repeatP7 - Samples 1 to 14: Sample 13 Acquiring

Spectrum Chromatogram Map Edit Samples

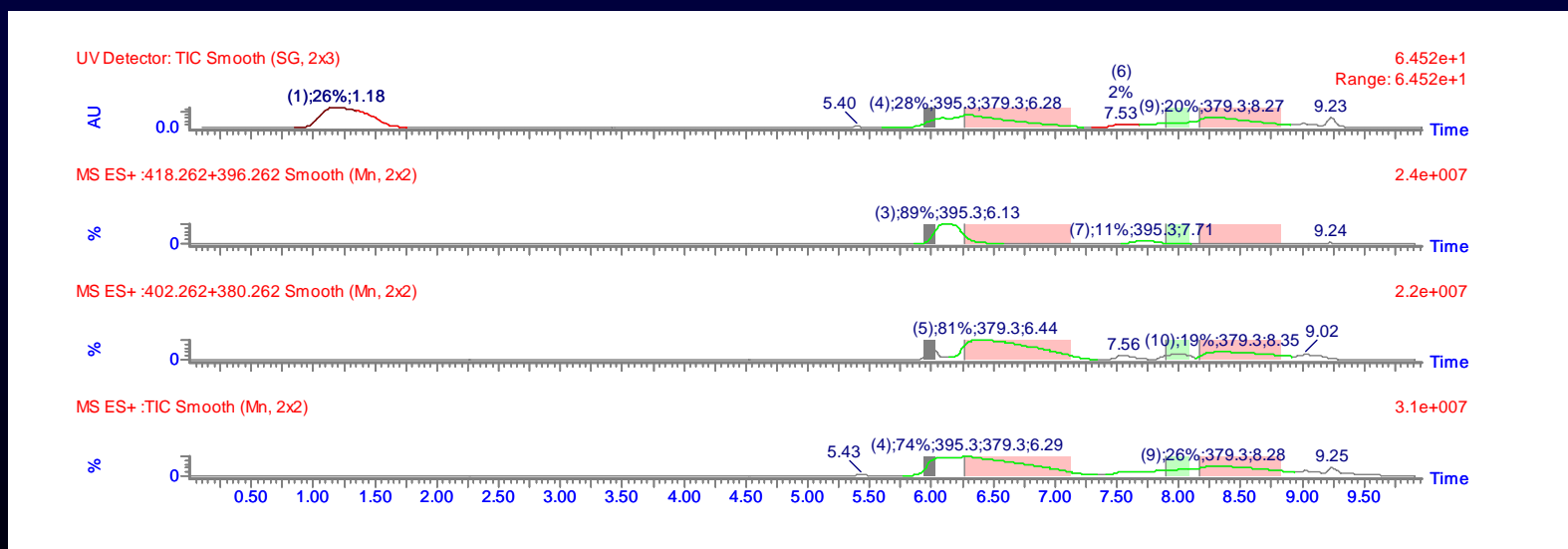
	File Name	File Text	MS File	Inlet ...	Fraction File	Bottle	Inject V...	Mass A	Mass B	Fraction Trigg...	Fra...	Process	Parameter File
1	GCR-2301-125-010		100to800_10min	0to2...	5M_35ML	5,2,16	300.000	341.1739	357.173...	Mass A		AutoPurify	Autopurify_Proc...
2	GCR-2301-125-011		100to800_10min	10to...	5M_35ML	5,2,17	300.000	403.2260	419.225...	Mass A		AutoPurify	Autopurify_Proc...
3	GCR-2301-125-012		100to800_10min	5to3...	5M_35ML	5,2,18	300.000	438.2631	454.263...	Mass A		AutoPurify	Autopurify_Proc...
4	GCR-2301-125-013		100to800_10min	5to3...	5M_35ML	5,2,19	300.000	325.2154	341.215...	Mass A		AutoPurify	Autopurify_Proc...
5	GCR-2301-125-014		100to800_10min	5to3...	5M_35ML	5,2,20	300.000	351.2310	367.231...	Mass A		AutoPurify	Autopurify_Proc...
6	GCR-2301-125-015		100to800_10min	10to...	5M_35ML	5,2,21	300.000	353.2467	369.246...	Mass A		AutoPurify	Autopurify_Proc...
7	GCR-2301-125-016		100to800_10min	5to3...	5M_35ML	5,2,22	300.000	365.1715	381.171...	Mass A		AutoPurify	Autopurify_Proc...
8	GCR-2301-125-018		100to800_10min	5to3...	5M_35ML	5,2,23	300.000	367.2260	383.225...	Mass A		AutoPurify	Autopurify_Proc...
9	GCR-2301-125-019		100to800_10min	5to3...	5M_35ML	5,2,24	300.000	381.2416	397.241...	Mass A		AutoPurify	Autopurify_Proc...
10	GCR-2301-125-020		100to800_10min	0to2...	5M_35ML	5,2,25	300.000	368.2212	384.2212	Mass A		AutoPurify	Autopurify_Proc...
11	GCR-2301-125-021		100to800_10min	10to...	5M_35ML	5,2,26	300.000	373.2154	389.215...	Mass A		AutoPurify	Autopurify_Proc...
12	GCR-2301-125-022		100to800_10min	5to3...	5M_35ML	5,2,27	300.000	391.2060	407.20597	Mass A		AutoPurify	Autopurify_Proc...
13	GCR-2301-125-023		100to800_10min	10to...	5M_35ML	5,2,28	300.000	403.2260	419.225...	Mass A		AutoPurify	Autopurify_Proc...
14	GCR-2301-125-024		100to800_10min	10to...	5M_35ML	5,2,29	300.000	387.2310	403.231...	Mass A		AutoPurify	Autopurify_Proc...

- Gradient window is an approximation of the optimal gradient.
- Selection based on retention time window.
- Method transfer from Acquity is challenging.

# From Generic to Narrow Gradients



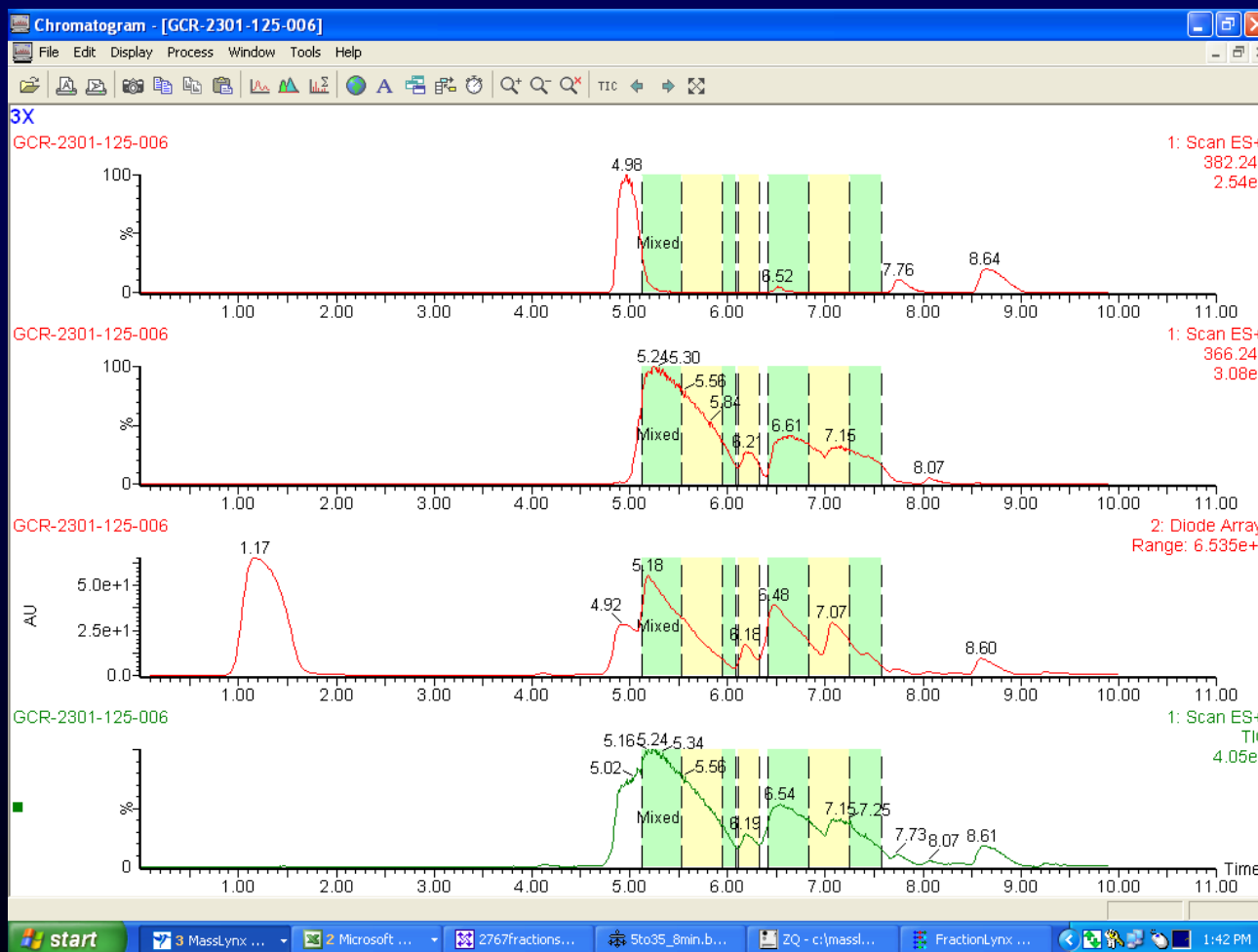
Acquity  
LC-MS  
"Generic"  
gradient



Preparative  
LC-MS

# Fractionlynx Boolean Operator

Mixed trigger: Mass A not Mass B

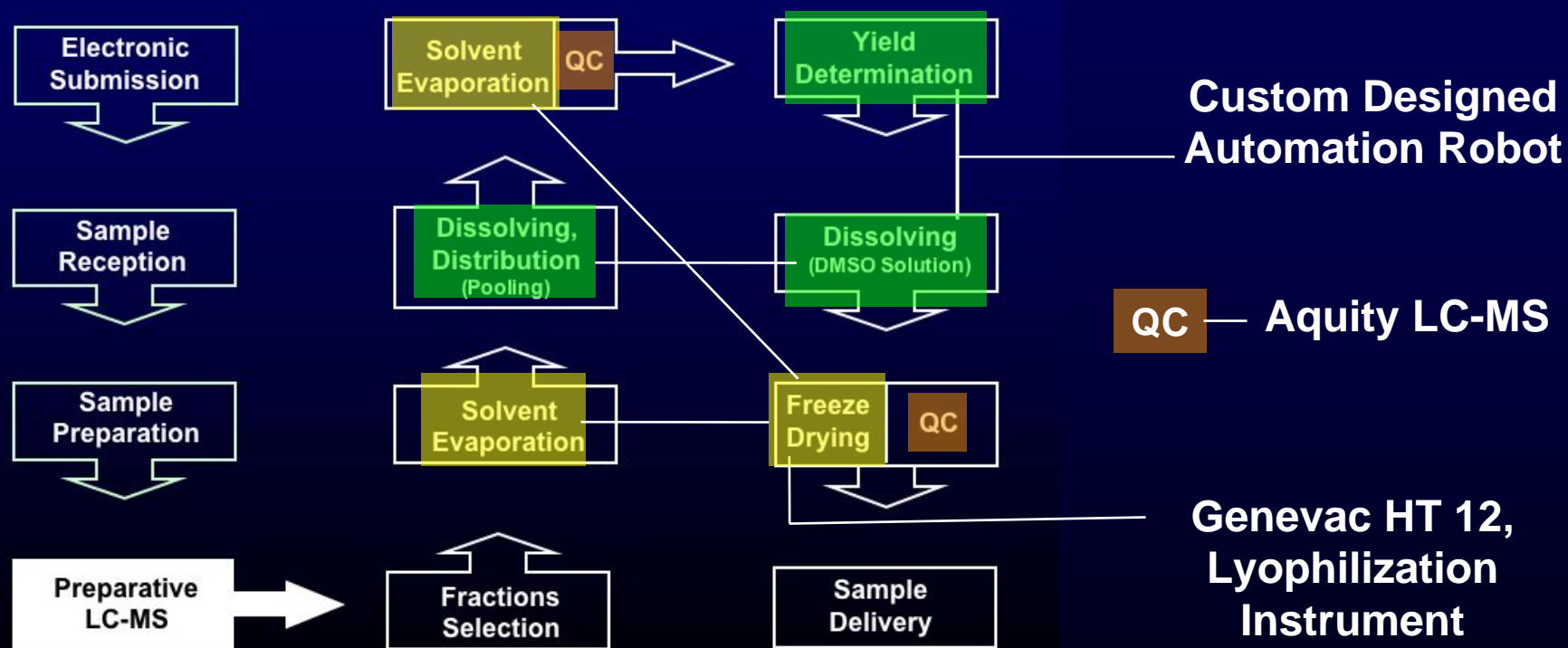


Mass B

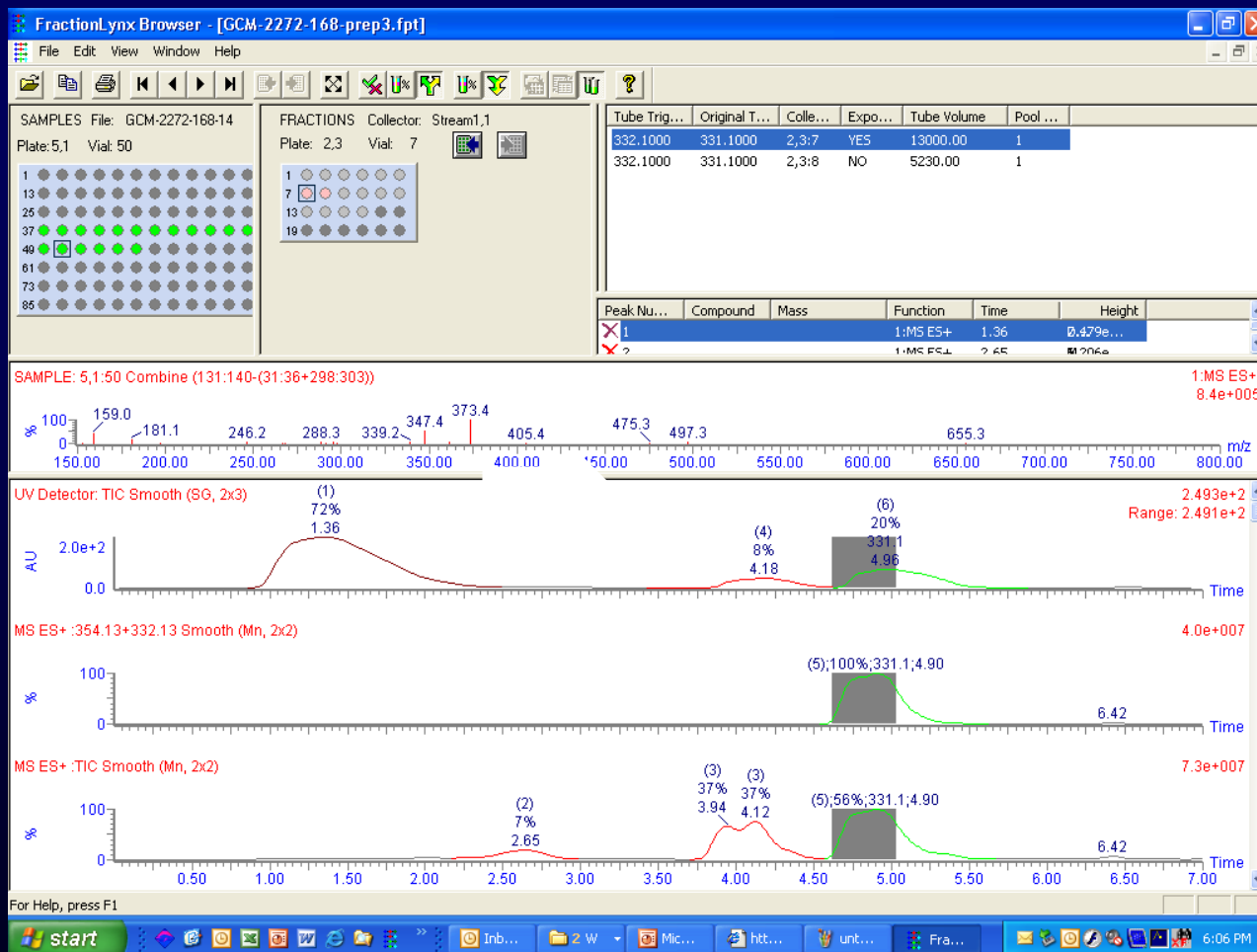
Mass A

# Post-Preparative Chromatography

## Samples Purification Workflow



# Fraction Selection



Fraction containing Product selected after manual review



Fraction list file containing pooling & combining information

# Fraction Tubes Handling

## Multitasker Robotic Instrument

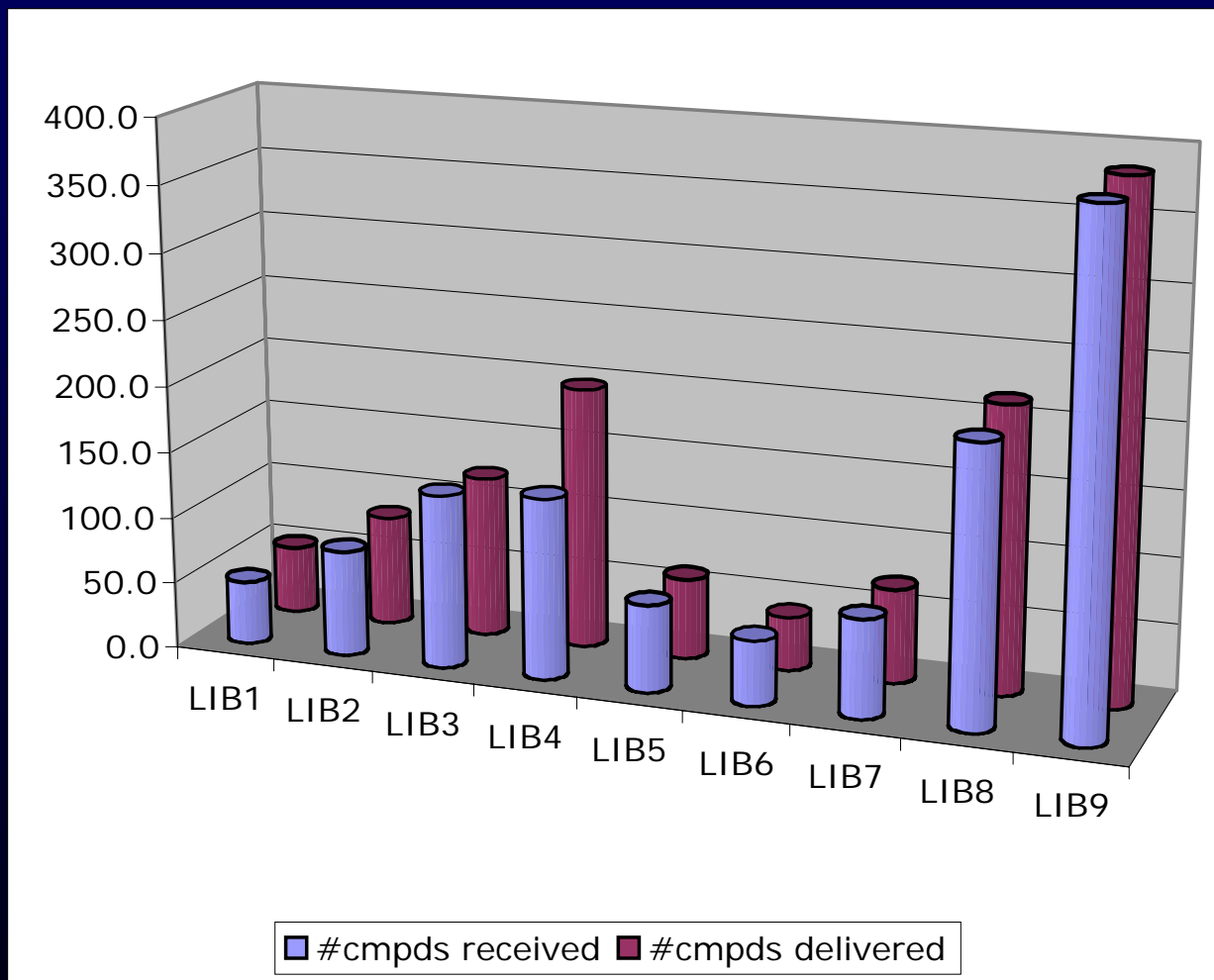


- 1D/2D barcodes reading.
- Weighing of different vial formats
- Solvent addition to tubes and transfer from tubes.
- Sonication and vortexing.
- Import of fraction's list file obtained from Fractionlynx via LIMS.

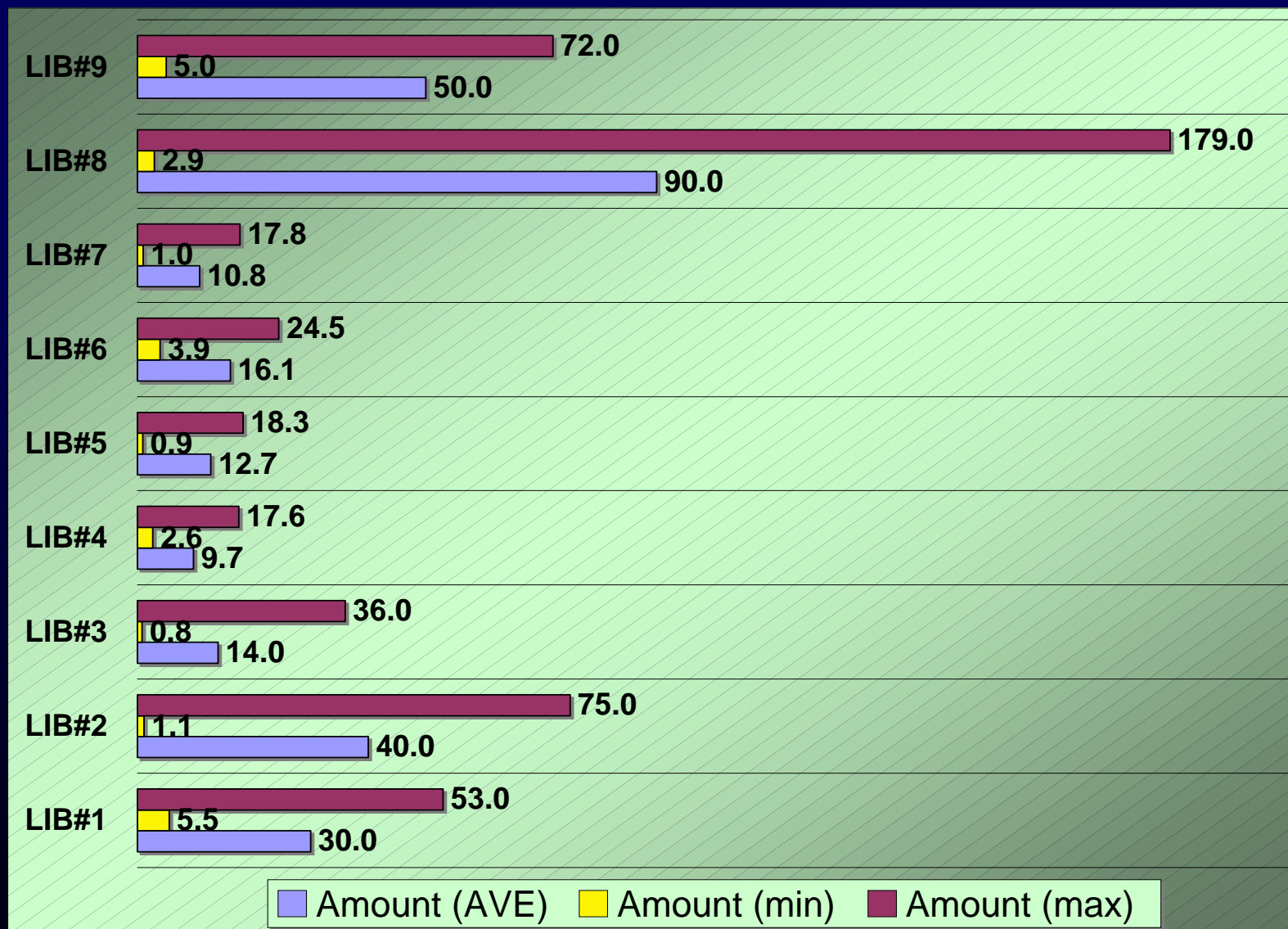


Solvent evaporation  
and/or lyophilization

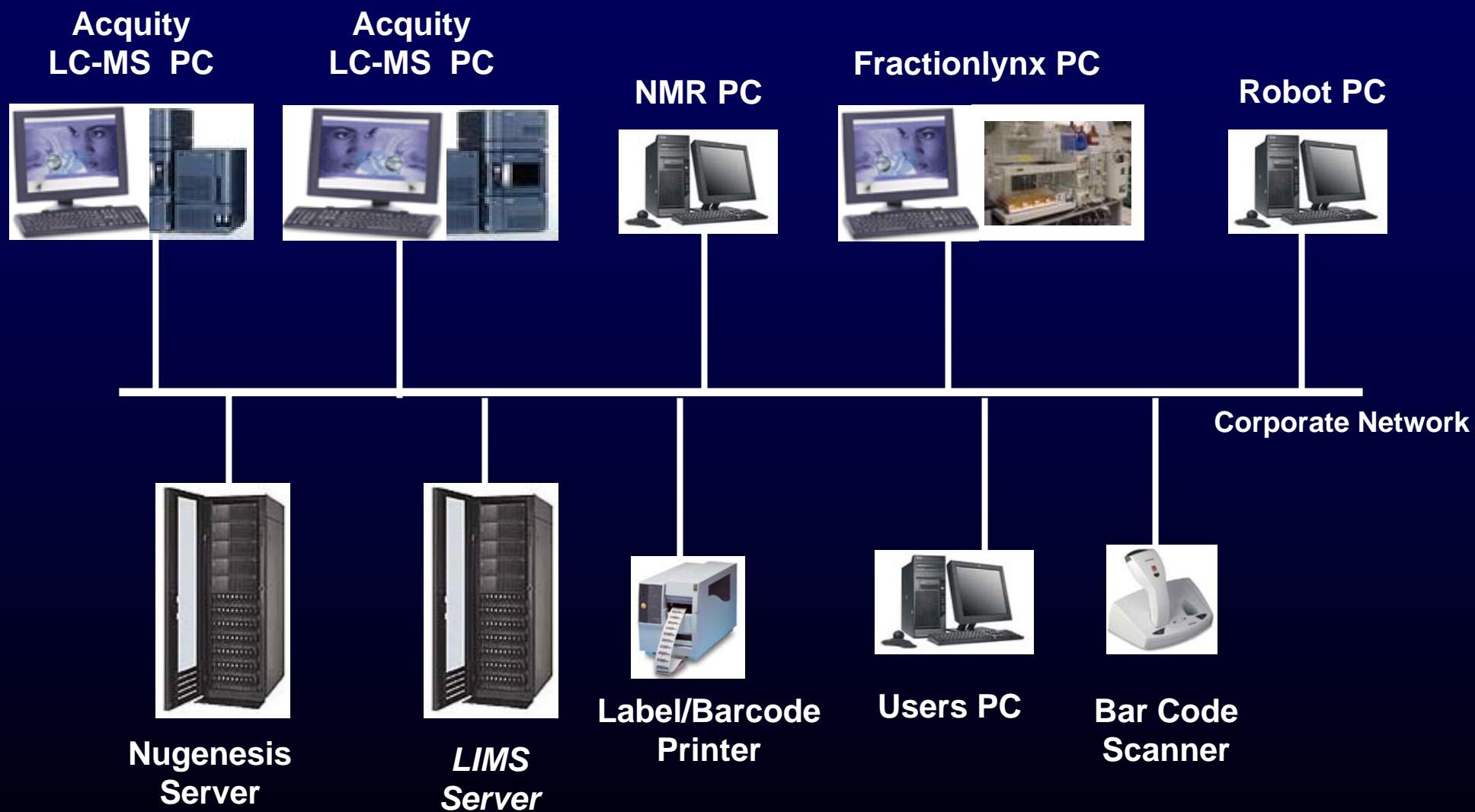
# Examples of Purified Libraries



# Amount Range of Purified Libraries

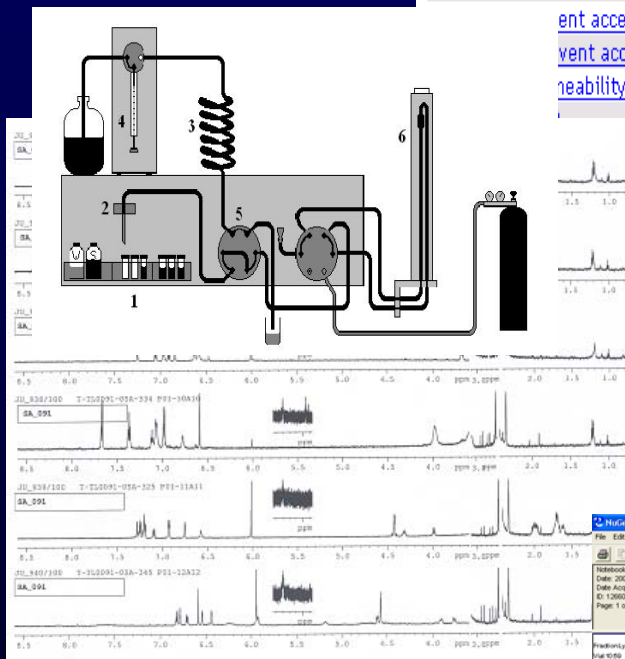


# Data Workflow and Archiving



# Data Archiving & Sharing

Property	Median	Acceptable Range	% In Range
<a href="#">Molecular weight</a>	432.6	132.0 - 550.0	98.3%
<a href="#">Octanol/water partition coefficient (log P(o/w))</a>	2.7	-1.5 - 5.2	96.2%
<a href="#">Number of rotatable bonds</a>	4.0	0.0 - 10.0	100.0%
<a href="#">Number of donor hydrogen bonds</a>	1.0	0.0 - 4.5	100.0%
<a href="#">Number of acceptor hydrogen bonds</a>	9.2	1.0 - 10.0	71.5%
<a href="#">Total accessible surface area</a>	91.7	3.0 - 260.0	100.0%
<a href="#">Polar accessible surface area</a>	308.2	0.0 - 460.0	90.5%
<a href="#">Lipophilicity Boehringer</a>	27.7	5.0 - 100.0	82.1%
<a href="#">ClogP</a>	-4.5	-6.0 - 0.0	81.8%
<a href="#">ClogS</a>	187.5	25.0 - 0.0	
<a href="#">MolLogP</a>	0.0	0.0	



**LC-MS, NMR,  
Calculated  
physico-chemical  
properties**

11Z\_LPLC\_MS In Drug Development Print Data on BATTUS - NuGenesis VISION - Microsoft Internet Explorer provided by Cephalon, Inc

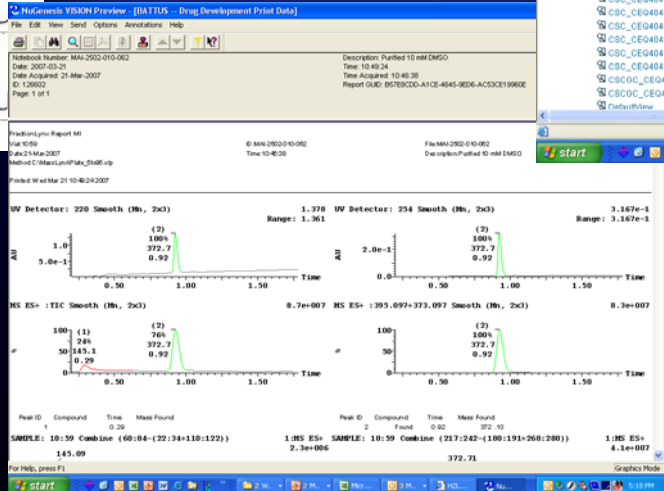
Server: BATTUS Print Data Project: Drug Development Print Data View: HGL\_LPLC\_MS User: grosse

Recently viewed

- BATTUS
- Drug Development Print Data
- HGL\_LPLC\_MS
- All Available
- Print Data
- BATTUS
- Drug Development Print Data
- Analytical
- Chromatation
- CRY0001FD
- CBC Agent Desktop
- CBC\_CE04020\_1
- CBC\_CE04020\_2
- CBC\_CE0403
- CBC\_CE0404
- CBC\_CE04040\_1
- CBC\_CE04040\_10
- CBC\_CE04040\_2
- CBC\_CE04040\_1
- CBC\_CE04040\_2
- CBCOC\_CE04041
- CBCOC\_CE0406
- PerformView

Select:	Notebook Number	Description	Date	Time	Date Acquired
1	RINGTA		2007-03-21	16:44:47	21-Mar-2007
2	GTAMINE		2007-03-21	16:29:33	21-Mar-2007
3	ZHQ		2007-03-21	16:26:47	21-Mar-2007
4	HRMEZ		2007-03-21	16:24:00	21-Mar-2007
5	GCR-2301-151-001		2007-03-21	14:05:20	21-Mar-2007
6	GCR-2301-149-001		2007-03-21	14:02:42	21-Mar-2007
7	MAI-2502-010-062	Purified 10 mM DMSO	2007-03-21	10:49:24	21-Mar-2007
8	MAI-2502-010-061	Purified 10 mM DMSO	2007-03-21	10:46:36	21-Mar-2007
9	MAI-2502-010-060	Purified 10 mM DMSO	2007-03-21	10:43:47	21-Mar-2007
10	MAI-2502-010-059	Purified 10 mM DMSO	2007-03-21	10:40:57	21-Mar-2007

NuGenesis SDMS



# Future Directions

- Streamline sample workflow process
- Refine autopurification methods, gradient prediction software integrated into Fractionlynx.
- Integration of barcoded fraction tubes into Fractionlynx.
- “No modifiers” chromatography.
- Final products as powders only.
- SFC-MS Separation as a complementary method.

**Waters  
Corporation**

John van Antwerp  
Laura Mulering  
Denise Heyburn  
Lisa McLaughlin  
Ronan Cleary  
Neil Conrad  
Angela Brady  
Marc Nobles  
Jo-Ann Jablonski  
Denise Kent  
Dennis Driussi  
Zack Blaszcak  
Blake Mcrudy



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