Modern Open Access Analytical Systems

Truly Automated Purification

Smriti Khera, Ph.D.
Pharmaceutical Discovery Marketing Manager
Life Science Group

smriti_khera@agilent.com
General Workflow for Chemical Matter Generation

- Synthesis: Reaction Monitoring, Structure Confirmation
- Purification: Analysis, Chiral/Achiral, Preparative/Semi-preparative Purification
- Activity and Properties: Biological activity, Physio-chemical properties, ADME

Optimize

Scale-Up

PASS

FAIL
General Workflow for Chemical Matter Generation

- **Synthesis**
  - Reaction Monitoring
  - Structure Confirmation

- **Purification**
  - Analysis
  - Chiral/Achiral
  - Preparative/Semi-preparative Purification

- **Activity and Properties**
  - Biological activity
  - Physio-chemical properties
  - ADME

- **Scale-Up**

- **Optimize**

- **PASS**

- **FAIL**
Reaction Monitoring Workflow

Synthetic chemists have limited MS expertise and need ease of use and rapid reporting of results.

MassHunter Walkup

Reliable, rugged LC/MS in central lab
Agilent LC/MS system

Did I make the right compound?

Molecular weight and purity confirmed!

Chemists and managers need fast browsing and reporting at desk.

Analytical Studio Reviewer

Agilent Technologies
OA LC/MS: The 90s

After implementation of OA at a Wyeth PA site, within the first 2 years, the total number of open-access analyses increased by ~142%.

In fact, the number of LC/MS analyses increased by >285%. This is attributed to the fact that the chemists began using the LC/MS data as a tool to monitor reactions and also to check final product integrity and purity.

(Similar reports from: Pullen et al. JASMAS, 1995; Taylor et al. JASMAS, 1994)

Mallis et al., J. Mass Spectrom. 2002
MassHunter Walkup LC/MS for Reaction Monitoring: As easy as 1-2-3

1. **Login**
   - Enter username and number samples to be run.

2. **Choose Method**
   - Enter sample information and select from a list of available methods.

3. **Place Sample**
   - Place sample in the position as directed by the software.

**Receive Report**

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*Agilent Technologies*
MassHunter Walkup Software: Easy Administration

Allows lab managers to maintain a multi-instrument LC and LC/MS facility; while users untrained in chromatography or mass spectrometry can run their own samples and still have high system up-time

- Remote administration with remote lab monitoring
- Easy and integrated administration of users and setup of new users
- Simple, customizable sample submission
- Results e-mailed to submitter
General Workflow for Chemical Matter Generation

- Synthesis
  - Reaction Monitoring
  - Structure Confirmation

- Purification
  - Analysis
  - Chiral/Achiral
  - Preparative/Semi-preparative Purification

- Activity and Properties
  - Biological activity
  - Physiochemical properties
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- Scale-Up

- Optimize

PASS
FAIL

Optimize

Scale-Up
The Open Access users see a streamlined, easy-to-use interface for sample submission, enabling them to quickly request a single experiment, or an entire study. VnmrJ acquisition software automatically accounts for the details of each sample and solvent, optimizing all measurement parameters as needed on the fly, so that all measurements deliver useful results.

- Easy Administration: customizable user configuration
- The VnmrJ Study Q
- Dynamic parameter inheritance
- Automated sample-dependent instrument optimization

General Workflow for Chemical Matter Generation

- **Synthesis**
  - Reaction Monitoring
  - Structure Confirmation

- **Purification**
  - Analysis
  - Chiral/Achiral
  - Preparative/Semi-preparative Purification

- **Activity and Properties**
  - Biological activity
  - Physio-chemical properties
  - ADME

- **Scale-Up**

- **Amenable to Open Access?**
  - Amenable to Open Access?
  - Optimize

- **PASS**
- **FAIL**
“Purification of compounds typically takes a medicinal chemist from 25 to 50% of their laboratory time” (DDT, 2008)
“Often, despite training coupled with experience, the most expedient approach is to centralize the practice, as Neurocine’s John Harman discovered. He found open-access practice for purification generally inefficient. When deciding how to proceed, he and his team ignored instrument usage time because “the cost of a machine is nothing compared to, unfortunately, the intangible cost of time.” After analyzing runs for a year, he found chemists were successful in purifying their molecules only about 40% of the time. Yet according to the chromatography, they should have been successful 70% of the time. Thus, the chemists were essentially throwing away 30% of their samples simply because they were not preparing them properly, choosing the wrong gradient, or setting the wrong threshold.” (LCGC 2009)

The Agilent Automated Purification System takes guess work out of Open Access Purification!
Previous Attempts at Automating Purification

Target for isolation

5-10% B
10-15% B
15-25% B
25-35% B
40-60% B
65-80% B

Retention Time [min]
Automated Purification Software

On the fly generation of focused gradients for your target molecule

Target for isolation

Focused Gradient

Generic Gradient

Result:
- Solvent savings
- Resolution increased on target peak
Automated Purification

Crude Sample & Product information

Analytical Confirmation

Automated Purification

Purity Evaluation, Fraction Pooling

Purity Determination

Archive Or/And Screening
**Coming Up:**

**Dr. Andreas Tei,**
Preparative HPLC Product Manager
Agilent Technologies, Inc.

*Overview of the Automated Purification System*

*How it works*

**Dr. Fabrizio Giordanetto,**
Director Medicinal Chemistry,
Taros Chemicals

*Overview of Taros chemical synthesis and purification workflow*

*Automated Purification in library and high-throughput operation*

**Acknowledgements:**

Helmut Schulenberg-Schell and Pierre Penduff, Agilent Technologies
The Purification Workflow

The workflow from the crude sample to the purified fraction

Crude Product + Sample Information → Target Confirmation + Analytical Scouting → Purification → Purity Determination Collected Fractions → Archive or / and Screening → Purity Determination Pooled Fractions → Fraction Pooling Reformatting
Manual Approach

Analytical Scouting

Get all relevant chromatographic information from your sample

Monitored wavelength: 254 nm

Generic Gradient:
- Flow: 1 ml/min
- Injection: 0.5 µl

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>5%</td>
</tr>
<tr>
<td>0.10</td>
<td>5%</td>
</tr>
<tr>
<td>1.60</td>
<td>95%</td>
</tr>
<tr>
<td>2.00</td>
<td>95%</td>
</tr>
</tbody>
</table>

Unresolved target compound by using a generic gradient
Manual Approach
Focussed Gradient Generation
Increase Chromatographic Resolution

Monitored wavelength: 254 nm

Focused Gradient:
Flow: 1 ml/min
Injection: 0.5 µl

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>5%</td>
</tr>
<tr>
<td>0.10</td>
<td>5%</td>
</tr>
<tr>
<td><strong>0.11</strong></td>
<td><strong>25%</strong></td>
</tr>
<tr>
<td><strong>0.61</strong></td>
<td><strong>35%</strong></td>
</tr>
<tr>
<td>0.62</td>
<td>95%</td>
</tr>
<tr>
<td>1.00</td>
<td>95%</td>
</tr>
</tbody>
</table>

Baseline separation after application of a focused gradient
Scaling Up From Analytical To Prep Columns

Calculate the flow rate, the gradient profile and the injection volume for purification

Goal: Keep the resolution and the performance achieved on the analytical system during the transfer on the preparative system

Formulas:

Flow rate calculation:

$$Flow_{PREP} = Flow_{ANA} \times \left( \frac{D_{PREP}}{D_{ANA}} \right)^2 \times \left( \frac{D_{part,ANA}}{D_{part,PREP}} \right)$$  \hspace{1cm} (a)

Isocratic hold:

$$T_{ini,PREP} = \left( T_{ini,ANA} + \frac{Dwell_{ANA}}{Flow_{ANA}} \right) \times \left( \frac{L_{PREP,col}}{L_{ANA,col}} \right) \times \left( \frac{D_{part,PREP}}{D_{part,ANA}} \right) - \frac{Dwell_{PREP}}{Flow_{PREP}}$$  \hspace{1cm} (b)

Segment duration:

$$T_{grad,PREP} = T_{grad,ANA} \times \left( \frac{L_{PREP,col}}{L_{ANA,col}} \right) \times \left( \frac{D_{part,PREP}}{D_{part,ANA}} \right)$$  \hspace{1cm} (c)

Injection Volume:

$$V_{inj,PREP} = V_{inj,ANA} \times \left( \frac{L_{col,PREP} \times D_{col,PREP}^2}{L_{col,ANA} \times D_{col,ANA}^2} \right)$$  \hspace{1cm} (d)

Guidelines for the use of UHPLC Instruments. Requirements for UHPLC instruments, method development in UHPLC and method transfer from regular HPLC to UHPLC

Dr. Davy Guillarme, Prof. Jean-Luc Veuthey
Purify The Sample, Get Your Fraction Results
With a successful method transfer from analytical – to- prep

Scale Up parameters:
- From 1.8 µm to 5 µm particle sizes columns
- From 153 µl to 3250 µl of dwell volume
- From 2.5 µl to 764 µl injection

Results:
- Collected target peak with 98% purity and 93% recovery
- Runtime gain: 12 min (generic gradient length 26 min)
- Solvent saving: 47% compared with linear gradient

Analytical Resolution = 1.03

Focused Gradient:
Flow: 1 ml/min
Injection: 2.5 µl

Time (min) %B
0.00 5%
0.10 5%
0.11 25%
0.61 35%
0.62 95%
1.00 95%

Preparative Resolution = 1.09

Focused Gradient:
Flow: 25 ml/min
Injection: 764 µl

Time (min) %B
0.00 5%
2.96 5%
2.97 25%
9.09 35%
9.10 95%
14.00 95%
Automated Purification Solution

In a nutshell – what is it?

➢ True Automated Purification – no manual purification method development

• A scalable, workflow-focused purification solution, built on 1200 Infinity Series modules with “Easy-Prep” and “Expert” mode to adapt to user knowledge & needs including automated delay volume calibration.

• Support of automated purification workflows from the import and export of sample sequences, re-analysis and re-formulation of collected fractions.

• Unattended purification with maximum purity and recovery using focused gradients tailored to each target compound calculated by a mathematical algorithm.

• Available for combined or separate LC/UV or LC/MS based systems.
Combined Analytical & Preparative Agilent
1260 LC-MS Purification System
Combined Agilent 1260 LC-MS Purification System

Analytical column: Zorbax SB C18, 4.6 x 50 mm, 5μ
Prep column: Zorbax SB C18, 21.2 x 150 mm, 5μ
Workflow on Separated Systems

Crude Sample & Target Compound Information: molecular formula/mass

Archive or / and Screening

Analytical Target Confirmation & Scouting for Focused Gradient Calculation

Purity Determination Pooled Fractions

Liquid Handler Fraction Pooling & Solvent Removal

Purification & Fraction Collection

Purity Review Collected Fractions Or Reanalysis

Workflow on Separated Systems

Agilent Confidential
Automated Purification Software in EasyPrep Mode

A simplified purification workflow

A reduced number of tabs will simplify the workflow in EasyPrep Mode!

1. Analyze the crude mix on a 1290 UHPLC – MS or 1260 System with Walk up SW
2. Submit a single sample or upload a sequence table for a plate/rack
3. Upload your analytical results at the purification system
4. Start the data process
5. Optional: Review analytical results
6. Collect fractions
7. Submit the fraction results for reanalysis or to a liquid handler
Automated Purification Software in “EasyPrep” Mode

How do we meet the needs of all non expert chromatographers!

Upload your analytical results

A reduced number of tabs will simplify the workflow in EasyPrep Mode!

Review chromatographic and spectral data

Fractions Results Browser
Define Analytical & Prep Systems In Expert Mode
Enter dwell and delay volumes

Analytical 1260 Infinity Binary LCMS System
Preparative 1260 Infinity PS LCMS System
Define Analytical & Prep Columns In Expert Mode
Enter column dimensions

<table>
<thead>
<tr>
<th>Column Details</th>
<th>Analytical System</th>
<th>Preparative System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Analytical Column</td>
<td>Preparative column</td>
</tr>
<tr>
<td>Length</td>
<td>100 mm</td>
<td>100 mm</td>
</tr>
<tr>
<td>Diameter</td>
<td>4.6 mm</td>
<td>21.2 mm</td>
</tr>
<tr>
<td>Particle Size</td>
<td>3.5 μm</td>
<td>5.0 μm</td>
</tr>
<tr>
<td>Void volume</td>
<td>0.881 ml</td>
<td>18.708 ml</td>
</tr>
<tr>
<td>Porosity</td>
<td>53.0 %</td>
<td>53.0 %</td>
</tr>
</tbody>
</table>
Optimize The Default Gradient Profile For Your Library

The default has been calculated based on the selected set of columns and flow rates.
Save Your System Template
Save All System Parameters In a Template
Apply a Stored Template To Purify Your Batch
## Upload An Analytical Result Set

### Analytical Run

Select System:
- Delay Volumes
- Columns
- Ion Species

#### Analytical Run

Specify that the analytical run has to be submitted or that analytical data has to be imported.

- **Submit analytical run**

- **Read analytical data**

  ```
  C:\Chem32\Purify\Tasks\Task 009\AnalyticalResults\131203_ANALYTICAL_SEQ_01 2013-12-03 18:36:38
  ```

<table>
<thead>
<tr>
<th>Process?</th>
<th>Valid</th>
<th>Sample</th>
<th>Sample type</th>
<th>Location</th>
<th>In volume [μL]</th>
<th>Target formula</th>
<th>Target mass [Da]</th>
<th>Acquisition method</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔</td>
<td>✔</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>1</td>
<td>5.000</td>
<td>154.0</td>
<td></td>
</tr>
<tr>
<td>✔</td>
<td>✔</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>1</td>
<td>5.000</td>
<td>152.0</td>
<td></td>
</tr>
<tr>
<td>✔</td>
<td>✔</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>1</td>
<td>5.000</td>
<td>264.0</td>
<td></td>
</tr>
<tr>
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<td>✔</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
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<td>5.000</td>
<td>310.0</td>
<td></td>
</tr>
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<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>1</td>
<td>5.000</td>
<td>180.0</td>
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<tr>
<td>✔</td>
<td>✔</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>1</td>
<td>5.000</td>
<td>228.0</td>
<td></td>
</tr>
</tbody>
</table>
Define the sample location, the prep injection volumes. Process the data: Hit the run sample button!

### Setup the sequence table of the preparative run

<table>
<thead>
<tr>
<th>Valid</th>
<th>Sample</th>
<th>Sample type</th>
<th>Location (Analytical)</th>
<th>Location (Preparative)</th>
<th>Inj volume [μl]</th>
<th>Target formula</th>
<th>Target mass [Da]</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>P1-C-01</td>
<td>750.000</td>
<td></td>
<td>154.0</td>
</tr>
<tr>
<td>✓</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>P1-C-02</td>
<td>750.000</td>
<td></td>
<td>152.0</td>
</tr>
<tr>
<td>✓</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>P1-C-03</td>
<td>750.000</td>
<td></td>
<td>264.0</td>
</tr>
<tr>
<td>✓</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>P1-C-04</td>
<td>750.000</td>
<td></td>
<td>310.0</td>
</tr>
<tr>
<td>✓</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>P1-C-05</td>
<td>750.000</td>
<td></td>
<td>180.0</td>
</tr>
<tr>
<td>✓</td>
<td>A</td>
<td>Sample</td>
<td>P1-C-01</td>
<td>P1-C-01</td>
<td>750.000</td>
<td></td>
<td>228.0</td>
</tr>
</tbody>
</table>

Run sample button
Identify other compounds of your sample by spectral data
Review calculated gradient profiles, optimize gradient profiles and integration parameters for each sample individually if required. Hit the run button and continue!
Review the purity of your collected fractions
Export the names and locations of the selected fractions or create a new sequence table for fraction reanalysis.
Reanalysis of Collected Fractions
Browse all results on one screen
Automated Purification Software “Expert Mode”

How do we meet chromatographers needs?

In “Expert Mode“ chromatographers can optimize all default parameters!
Automated Purification Solution

Summary

- True Automated Purification – no manual purification method development

  - A scalable, workflow-focused purification solution, built on 1200 Infinity Series modules with “Easy-Prep” and “Expert” mode to adapt to user knowledge & needs including automated delay volume calibration.

  - Support of automated purification workflows from the import and export of sample sequences, re-analysis and re-formulation of collected fractions

  - Unattended purification with maximum purity and recovery using focused gradients tailored to each target compound calculated by a mathematical algorithm

  - Available for combined or separate LC/UV or LC/MS based systems.
Screening Library Synthesis and Automated Purification

C&EN webinars, April 10 2014

Fabrizio Giordanetto PhD
Director, Medicinal Chemistry, Taros
Taros: Stability, Capability, Flexibility

as a leader in chemistry research, Taros provides valuable contract discovery, development, and manufacturing services to meet our clients’ needs

- Founded 1999 in Marburg
- Privately owned
- Based in Dortmund, Germany (BioMedizinZentrum)
- 50 employees (> 65% Ph.D.) in Germany, 30 employees in India
- >1500 sqm state of the art labs in Germany
- Global facilities on 2 continents
- More than 6,000 projects delivered
- More than 200 customers served
- Invented “TarosGate®”, the smart 24h/7 Project Management Software

- Taros is coordinating the Chemistry Consortium of the “European Lead Factory” drug discovery platform
Experience

Outstanding scientific track-record

- >160 years combined industrial organic chemistry experience
- >80 years active drug discovery experience from big pharmas & biotechs
  - eg. AstraZeneca, Millennium, Merck, Novartis, Organon, Pfizer, Roche, Takeda
- >10 Ph. I – III deliveries
- >90 patents
- >140 publications
- Wide-ranging experience in molecular intervention:

<table>
<thead>
<tr>
<th>Therapeutic Area</th>
<th>Target class</th>
<th>Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV/ Metabolism</td>
<td>Enzyme</td>
<td>Inhibitor</td>
</tr>
<tr>
<td>CNS</td>
<td>GPCR</td>
<td>Agonist</td>
</tr>
<tr>
<td>Fertility</td>
<td>Ion channel</td>
<td>Antagonist</td>
</tr>
<tr>
<td>Infection</td>
<td>Nuclear Hormone Receptor</td>
<td>Allosteric modulator</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Protein-Protein interface</td>
<td>Activator</td>
</tr>
<tr>
<td>Oncology</td>
<td>Phenotype</td>
<td>Stabiliser</td>
</tr>
<tr>
<td>Pain</td>
<td>Transporter</td>
<td></td>
</tr>
</tbody>
</table>
Collaborative Drug Discovery

Joint European Compound Library

EFPIA contribution (300,000 cpds)

Public contribution (up to 200,000 cpds)

uHTS Compound logistics

Hit triage Medicinal Chemistry

Molecular Targets

The research leading to these results has received from the Innovative Joint Undertaking under grant agreement n° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7 / 2007-2013) and EFPIA companies in kind contribution.
Compound library criteria

Novelty
Rational/Innovation
Synthetic tractability
Diversity potential,
- libraries should yield 50-500 final compounds
Molecular properties, structural features

Ongoing libraries

Comparison between ELF library and widely used, commercial HTS screening libraries at a scaffold level (main source of novelty, main chemistry challenge)

ELF:
- Accepted ELF libraries, as described by the scaffolds (N=178)

<table>
<thead>
<tr>
<th>No. of Steps</th>
<th>Min</th>
<th>Max</th>
<th>Avg</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>16</td>
<td>7</td>
<td>6-9</td>
</tr>
</tbody>
</table>

Commercial:
- From commercial sources, as described by the scaffolds (diversity-based pick N=2400)
Different from commercially available

Diversity points

% of scaffolds

fsp³

% of scaffolds

Stereocenters

% of scaffolds

Scaffold Largest Ring (HAC)

% of scaffolds

ELF libraries
Comm. HTS lib
Taros Challenge

Highly innovative libraries but synthetically more demanding
- Higher chemistry attrition
- Higher reagent costs

⇒ Maximise organic chemistry time (innovation, optimisation, problem solving) vs. other tasks (eg. purification)

Taros Goals

40,000 compounds over 4 years
10,000 compounds/year
44 compound/working day
880 compound/month
3-4 libraries/month

Add your chemistry/purification attrition coefficient
Overall workflow

Synthesis – work-up – analysis – reformatting – analysis
Target compound: >5 mg, >85% LC-MS purity
Automated Synthesis and Purification Workflow

Automated reagent handling – Parallel synthesis

Parallel clean Up – Purification – Reformating – Reanalysis
Taros Laboratory Setup

1. Pilot library block (24x crudes, after work-up)
   • Diversity coverage (eg. reactivity, steric hindrance, physicochemical properties)
2. uHPLC analysis for scouting of samples
3. HPLC purification
4. UHPLC analysis

UHPLC/MS:
Agilent 1290 Infinity LC & 6120 Quad LC/MS

Preparative HPLC:
Agilent 1260 Infinity LC & 6120 Quad LC/MS
Compound library Purification Results

1. Interpretation of the UHPLC analysis data and selection of the purification method

2. Purification Chromatogram and selection of the pure fractions

3. UHPLC Final Analysis
Results & Observations

60% of ramp up achieved

Analytical UHPLC/MS is ideal for separation of complex synthetic mixtures
  - Efficient, high throughput analysis-purification set-up

Automatic transfer of analytical results to purification systems eliminates manual work
  - Up to 40-50% chemistry time gained
  - Minimal learning curve for untrained operators
  - Straightforward implementation in work-flow
  - Convenient export of fraction information to liquid handler from purification software

Different chemistries require method optimization
  - Possible to back-calculate chemistry scale and library design
  - Possible to automatically collect reagent statistics for improved chemistry/design success
Time for Questions and Discussions