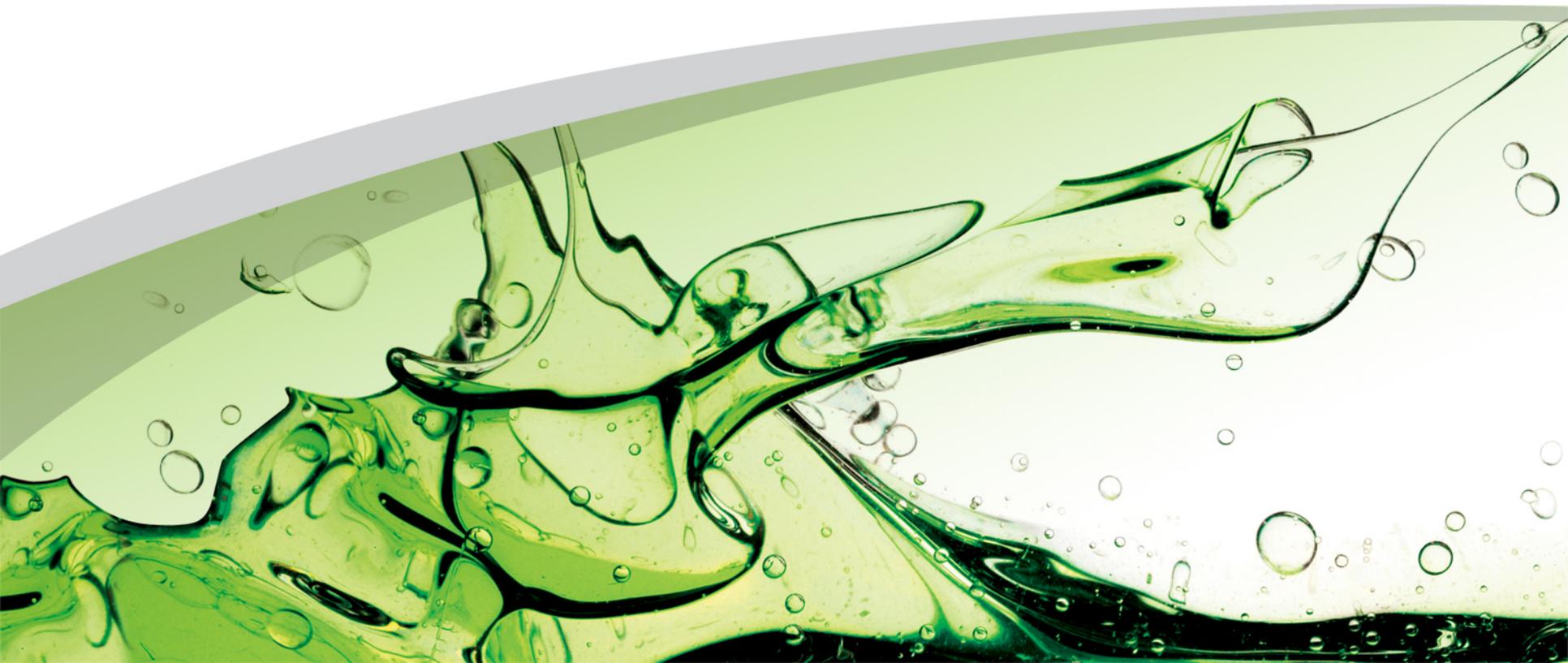




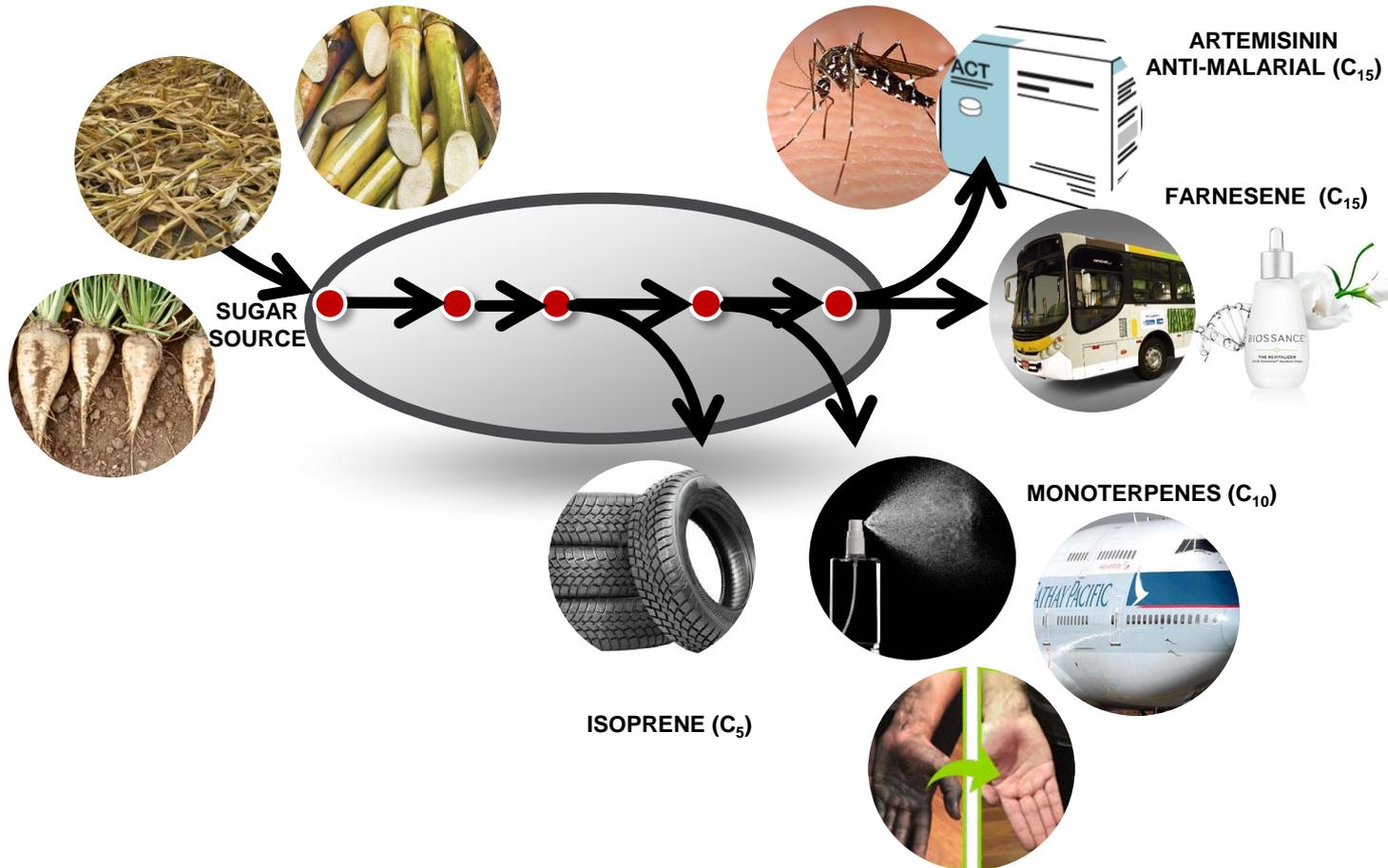
# High Throughput Small Molecule Detection

Skyline User Group Meeting 6/4/2017

Yang Zhang



# Amyris: from sugar to product



# DARPA M2K: from mg to kg in record time



**Year 1**  
Attempt to produce 450 molecules at mg scale

**Year 2**  
150 molecules at gm scale

**Year 3**  
50 molecules at g/L/h scale

**Year 4**  
Purify 1 Kg of 10 molecules



## Faster

reduce the time to market by 4X



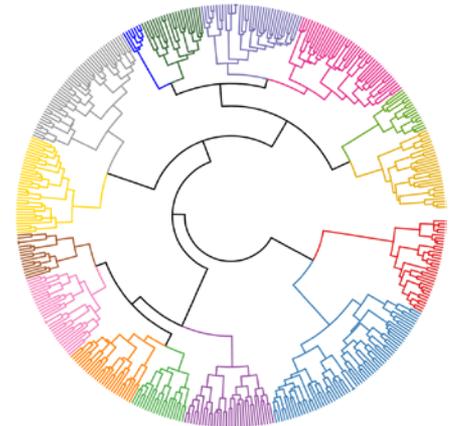
## Cheaper

reduce the cost of scale up by 10X



## Greater

increase molecule throughput by 20X



# Analytical workflow

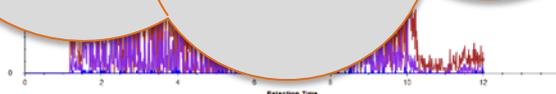
## Data Acquisition



## Data analysis

- Peak detection

Thousands of  
samples per  
week covering  
500+ molecules

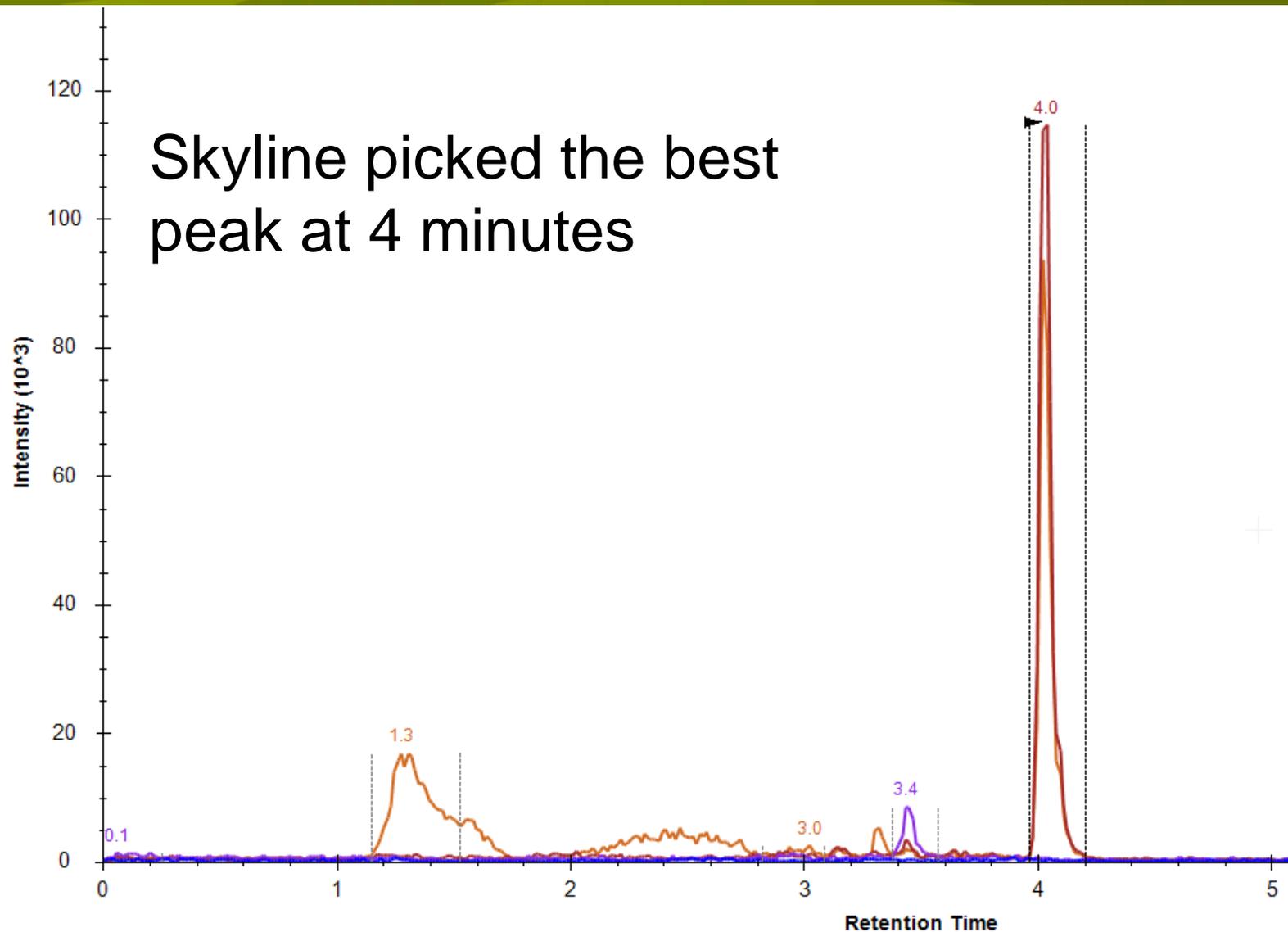


## Data uploading

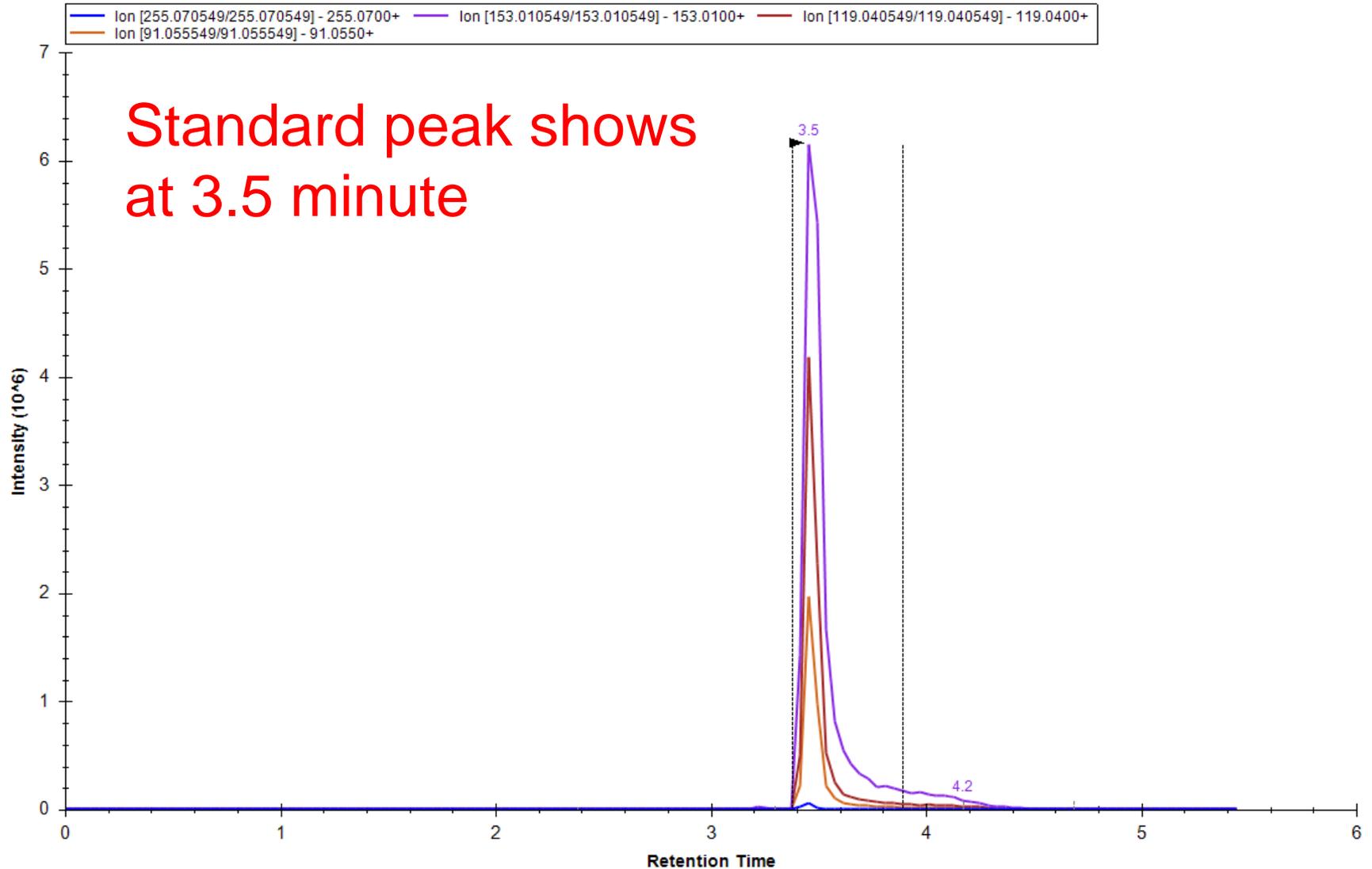
- Generate reports
- Save to database



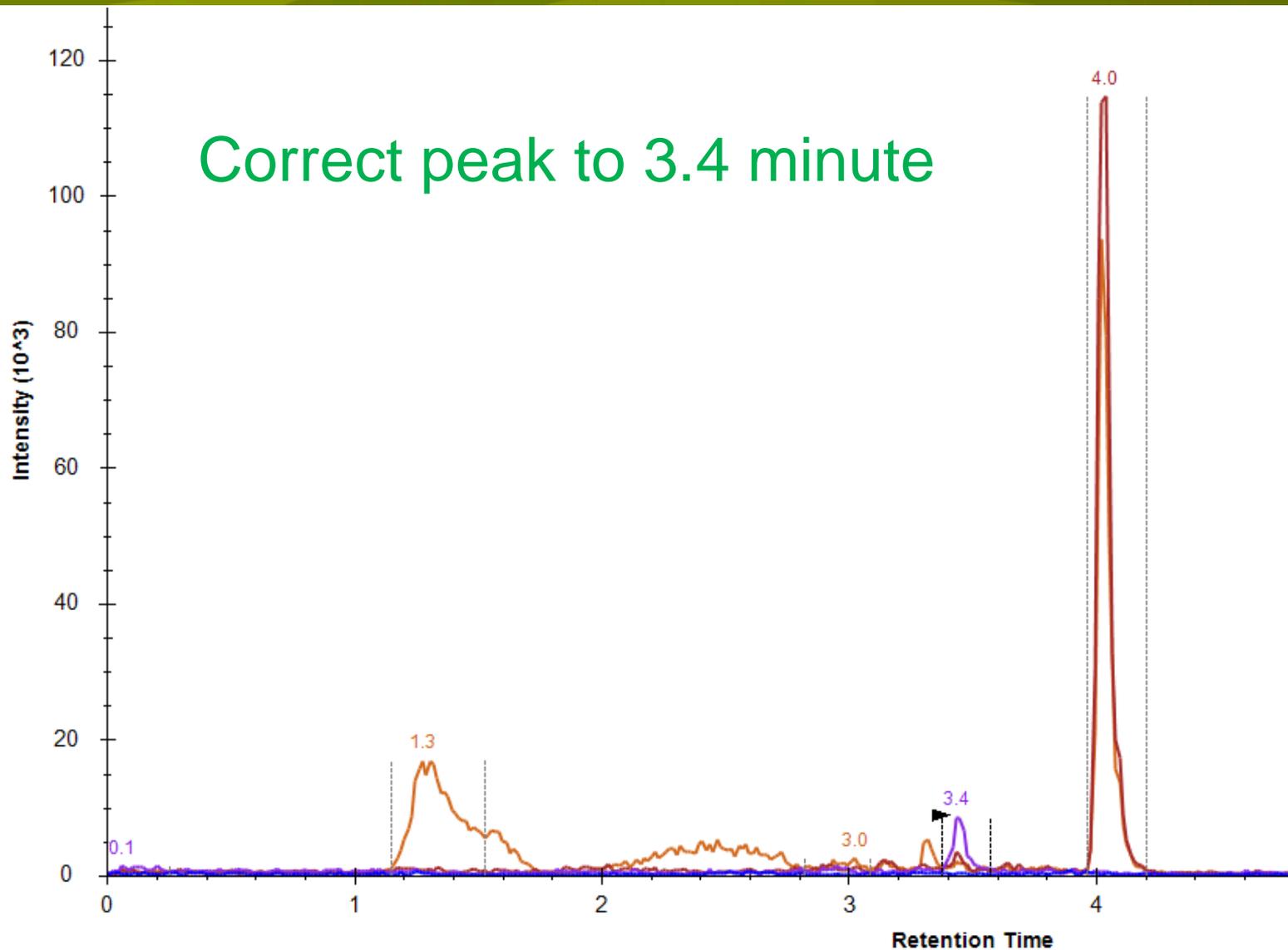
# Peak integration without RT correction



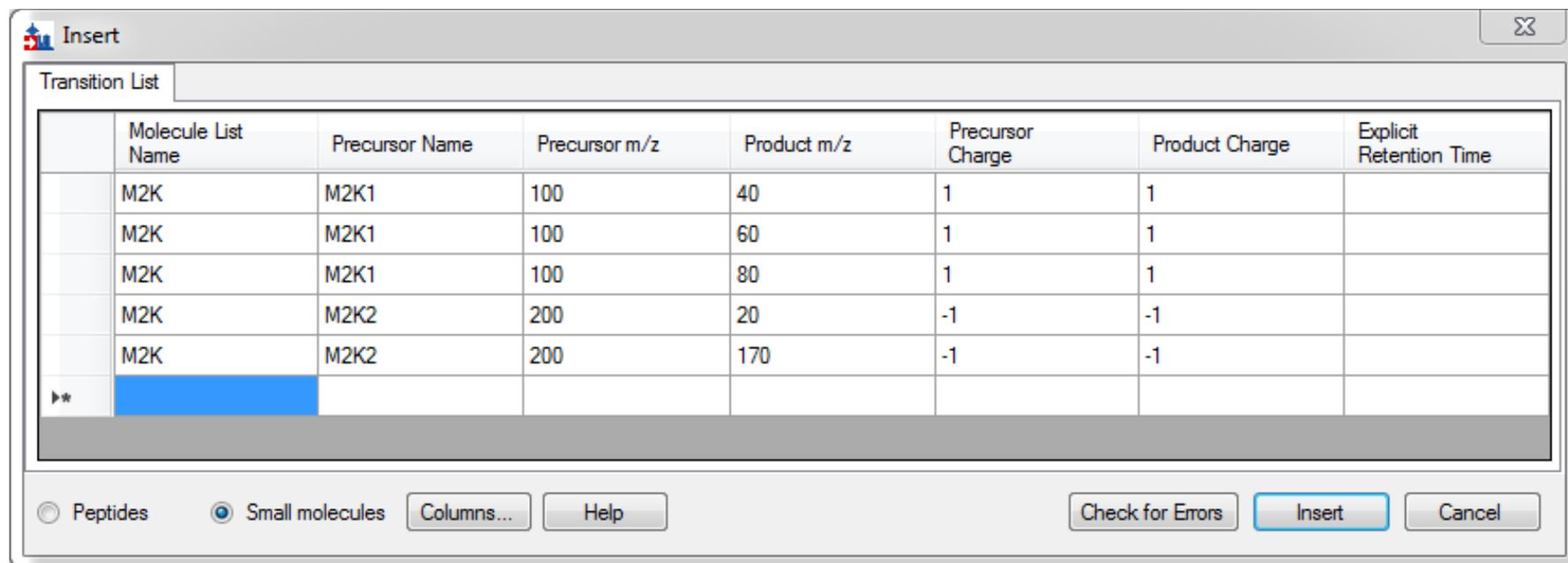
# Get RT from standard or best guess in sample



# Correct RT



# Skylinerunner + R: naïve peak integration



Insert

Transition List

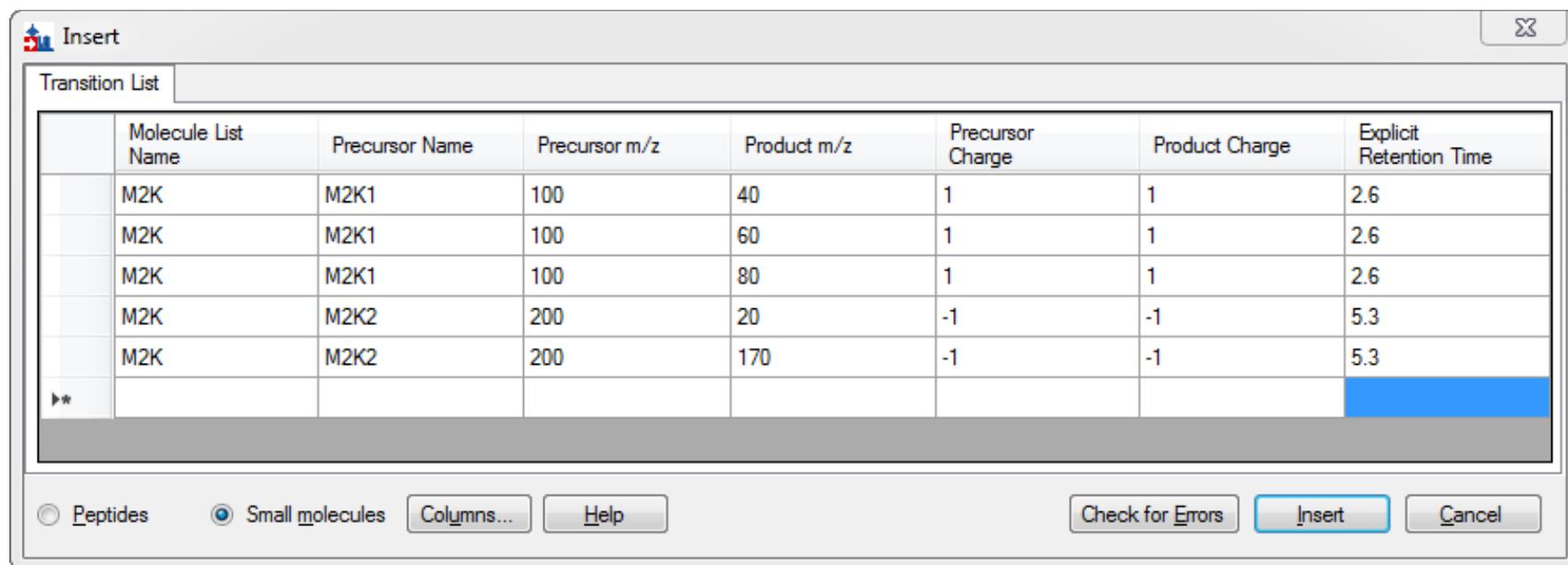
	Molecule List Name	Precursor Name	Precursor m/z	Product m/z	Precursor Charge	Product Charge	Explicit Retention Time
	M2K	M2K1	100	40	1	1	
	M2K	M2K1	100	60	1	1	
	M2K	M2K1	100	80	1	1	
	M2K	M2K2	200	20	-1	-1	
	M2K	M2K2	200	170	-1	-1	
▶▶							

Peptides  Small molecules

SkylineRunner.exe --in=skyline.sky  
--import-transition-list=transition.csv  
--out=skyline.sky

SkylineRunner.exe --in=skyline.sky --report-name=M2K  
--report-file=M2K.tsv --report-format=tsv  
--report-invariant --report-add=M2K.skyr  
--report-conflict-resolution=overwrite

# Skylinerunner + R: Retention time correction



The screenshot shows the 'Insert' dialog box in Skyline software. The 'Transition List' tab is active, displaying a table with the following data:

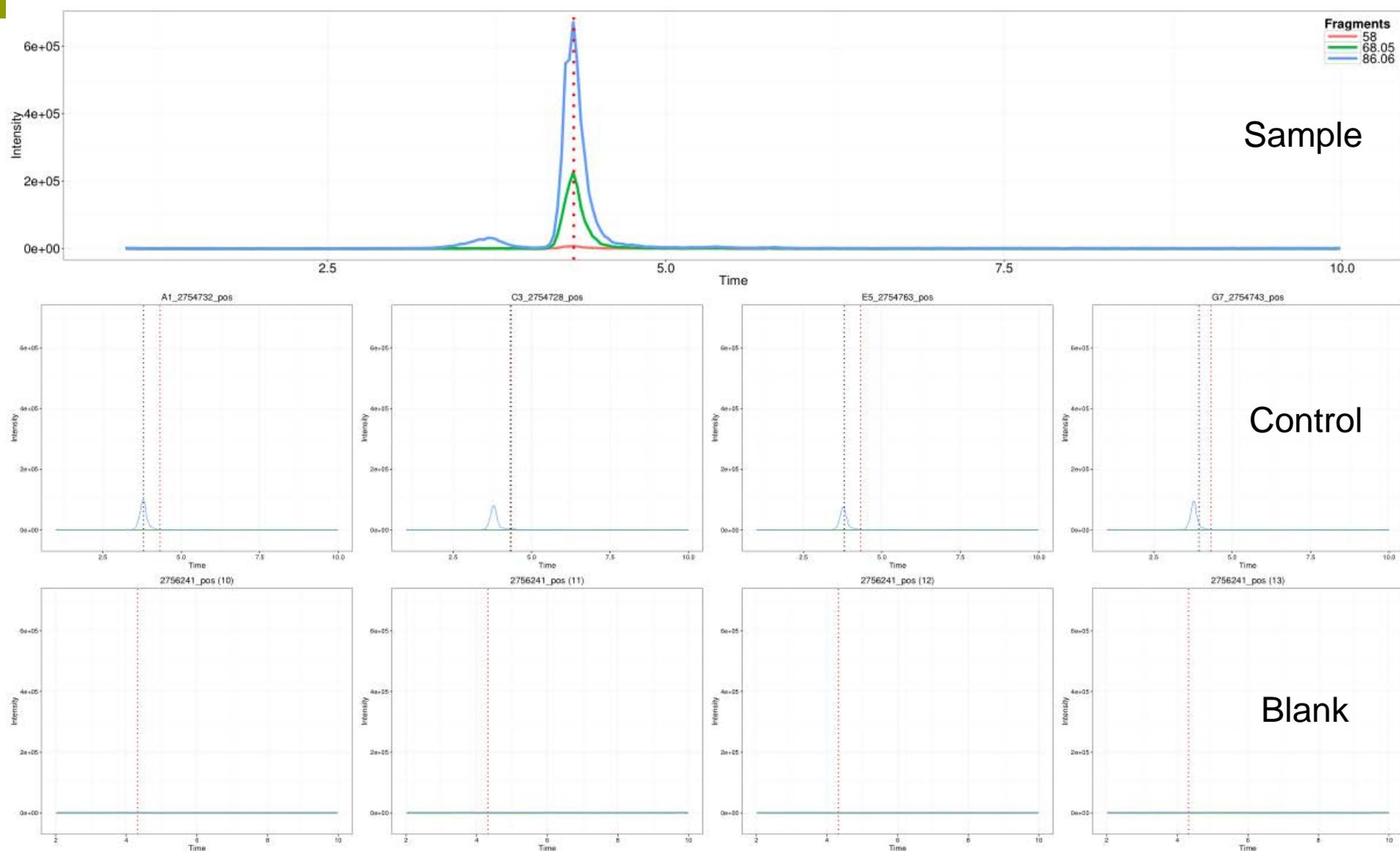
	Molecule List Name	Precursor Name	Precursor m/z	Product m/z	Precursor Charge	Product Charge	Explicit Retention Time
	M2K	M2K1	100	40	1	1	2.6
	M2K	M2K1	100	60	1	1	2.6
	M2K	M2K1	100	80	1	1	2.6
	M2K	M2K2	200	20	-1	-1	5.3
	M2K	M2K2	200	170	-1	-1	5.3
▶*							

At the bottom of the dialog, there are radio buttons for 'Peptides' and 'Small molecules' (selected), along with buttons for 'Columns...', 'Help', 'Check for Errors', 'Insert', and 'Cancel'.

```
SkylineRunner.exe --in=skyline.sky  
--import-transition-list=transition.csv  
--out=skyline.sky
```

```
SkylineRunner.exe --in=skyline.sky --report-name=M2K  
--report-file=M2K.tsv --report-format=tsv  
--report-invariant --report-add=M2K.skyr  
--report-conflict-resolution=overwrite
```

# Example output

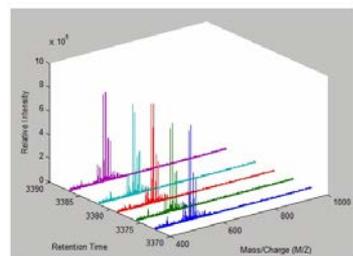


# Performance comparable to manual curation

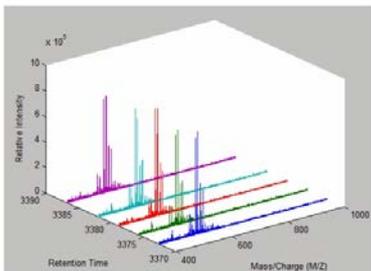
ID	Name	Assay	Manual Detected	Automatic Detected	Manual Hit	Automatic Hit	RT	Native	Notes
1	M2K1	HILIC_NEG					3.8	yes	
2	M2K2	HILIC_POS					4.4	no	
3	M2K3	HILIC_POS					4.75	yes	
4	M2K4	HILIC_NEG					5.55	yes	
5	M2K5	HILIC_POS					4.3	yes	
6	M2K6	HILIC_POS					4.67	yes	same as blank
7	M2K7	HILIC_POS					5.5	yes	
8	M2K8	HILIC_POS						no	
9	M2K9	HILIC_POS					4.5	yes	
10	M2K10	HILIC_POS					3.9	yes	
11	M2K11	HILIC_POS					4.42	yes	
12	M2K12	HILIC_POS						no	
13	M2K13	HILIC_NEG						no	
14	M2K14	HILIC_POS					5.03	yes	
15	M2K15	HILIC_NEG					4.6	yes	same as blank
16	M2K16	HILIC_NEG						no	
17	M2K17	HILIC_POS						yes	
18	M2K18	HILIC_POS					8.57	no	

# Hands-on time dropped from hours to minutes

Before:



Now:



# Acknowledgement

