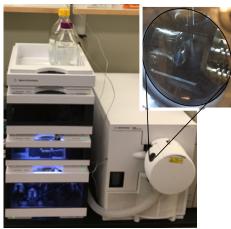
Agilent 6140 UHPLC-MS -Scott Virgil, California Institute of Technology May, 2016



The use of UHPLC technology in combination with fast Electrospray Mass Spectrometry has advanced the speed and efficiency of research in demanding fields of Organic Synthesis and Catalysis.

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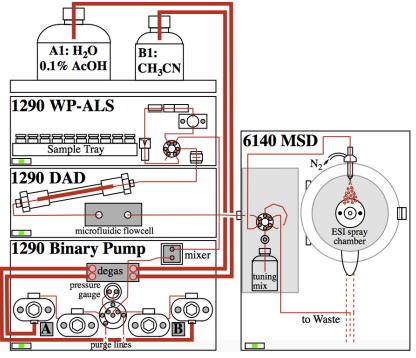
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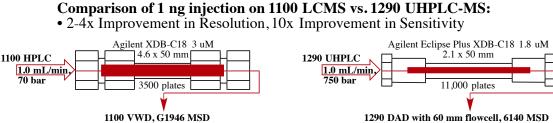
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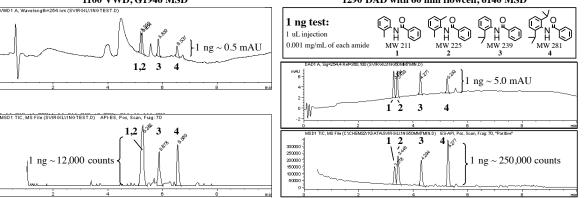
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In the above diagram, the key features of the Agilent 6140 UHPLC-MS instrument are shown:

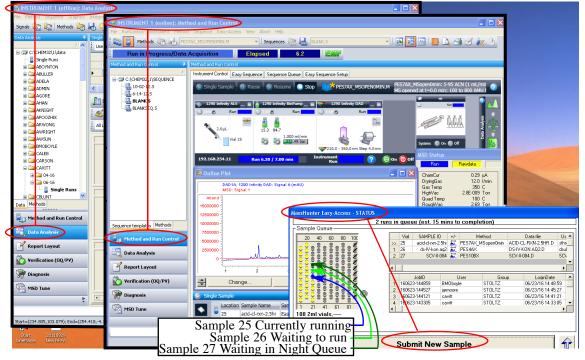
- 1. The **1290 Binary Pump** module delivers water and acetonitrile at pressures up to 1200 bar. It also includes a degasser, an auto-purge and a "Jet Weaver" microfluidic.
- 2. The **1290 Well-Plate Autosampler** accepts standard HPLC vial trays and 96-well plates.
- 3. The sample flows through a 2 μm filter to a 2.1 x 50 mm Eclipse Plus 1.8 μm particle size C18 column (part No. 959757-902) at a flow rate of 1 mL/minute and 700-800 bar.
- 4. The **1290 Diode Array Detector (DAD)** with a 60 mm path length microfluidic flowcell detects the absorbance a 6x improvement in sensitivity over standard 10 mm flowcells.
- 5. The **6140 MS Single Quad MS detector** with standard ESI source is specifically designed to provide fast data acquisition on a mass range of 50-1350 m/e range.





UHPLC-MS System Operation with Agilent Chemstation and Easy-Access

The Agilent UHPLC-MS is operated using an **Online Chemstation** window that runs the instrument and an **Offline Chemstation** window that is used for *data analysis*. In addition, **Agilent Easy-Access** software is used to efficiently handle multiple users in a walk-up format.



I. How to submit samples to the Easy Access queue.

- Hit "Submit New Sample" and enter your login account and password.
- Select the number of samples to submit and enter the Sample ID(s).

las	assHunter Easy-Access - Sample Data Input - SCV								
	User Login				Sample Data Input			ut	Sample Loading
Γ		SAMPLE ID		Method		Inj. Vol.	UV (nm)		▲
	1	SCV-III-084	PES10BX		-	1.00	254	F	xamples - <i>Correct</i>
	2	SCV-III-085	PES4AX_20	-95	-	1.20	272		
	3	SCV-III.087 *crude*	NES7CX		-	1.00	254	X	- Don't use . or * in Sample Name
	4	SCV-III-084	NES4AX_MS	SOPENOMIN	-	1.00	254	v	- Don't duplicate Sample Name
4	X - Don't auplicate Sample Name								
	Automatically copy down column								
		<u>B</u> a	ck			L	oad Sampl	es	Cancel

• Choose from the set of available Methods. Either **Positive** (PES) or **Negative** (NES) detection can be used in LCMS electrospray. However, positive electrospray ionizes a wider range of compounds. The methods have run times of 4,7 or 10 minute lengths. The "**AX**" methods have a **100-800 amu** mass range. For compounds that are above 190 amu, the "**BX**" has **190-800 amu** (which eliminates low MW impurities from the MS trace) and "**CX**" runs from **190-1350 amu**. If no other annotations are included, the gradient proceeds from 5% to 95% acetonitrile during the run time and the MS detector is bypassed until t = 0.5 min in order to minimize salts entering the MSD. For polar compounds, the _MSOpen0min can be used to open the MS at t = 0 min. Both the default Inj. Vol. and the UV_A wavelength may also be changed.

PES10BX	Pos. 10 min run, 5-95% ACN, 190-800 amu
PES4AX_20-95	Pos. 4 min run, 20-95% ACN, 100-800 amu
NES7CX_MSOpen0min	Neg. 7 min run, 5-95% ACN, 190-1350 amu, MS valve open 0 min

UHPLC-MS System Operation (continued)

II. Common Questions on Samples:

• How much sample do I need? The LCMS easily detects analytes at the 1 ng level which corresponds to a 1 μ L injection from a 0.001 mg/mL sample. However, most samples are about 0.1 mg/mL and give UV peaks ~ 500 mAu. Samples that are at or above 1 mg/mL are unnecessarily concentrated and will give poor data and diminish the life of the instrument.

• What solvents are OK? Acetonitrile is available for making up samples at the instrument. Most other standard organic solvents and water are suitable for sample submission. Chloroform and dichloromethane can affect the peak shapes if used at higher than 1 μ L injection volumes.

• How much solution do I need? The WP autosampler needs about 0.5 mL in a hplc vial as it draws from about ¹/₄ inch above the bottom. Vial inserts are available for smaller sample sizes.

• Do I need to filter my sample? No, unless the sample is obviously heterogeneous.

• **Can I submit "crude reaction mixtures"?** Yes. For reaction monitoring, usually a tip of a pipette diluted to 1 mL will provide a suitable sample.

• What about biological samples? Well, the worst thing for a C18 column to see is biological material including membrane proteins, phospholipids and biopolymers. These materials need to be removed from LCMS samples prior to submission or they will clog the column and degrade the peak shape of analytes.

Further notes on sample submission:

• When submitting more than 3 samples at a time, select the "Night Queue" to allow other samples to run first.

• For samples that are highly lipophilic, 20-95, 40-95 or 60-95 gradients can be used to improve separation and allow shorter run times.

III. Data Analysis

1. Auto-Print From Easy Access – check the box "Print Report" when submitting samples.

2. Snapshot – while a sample is running, Select "Snapshot" in the Offline Chemstation.

3. Load Signal to access previously run Data – then select file on C:/ or D:/ (old data) drive. If only one signal chromatogram is desired, it may be selected individually when loading.

IV. Tools for Data Analysis

When a data file is loaded, it will usually consist of three DAD chromatograms plus one MSD trace. They can be viewed individually or all together by selecting "All Loaded Signals". In addition, five buttons above the data provide different tools for Integration, Signal selection, etc.

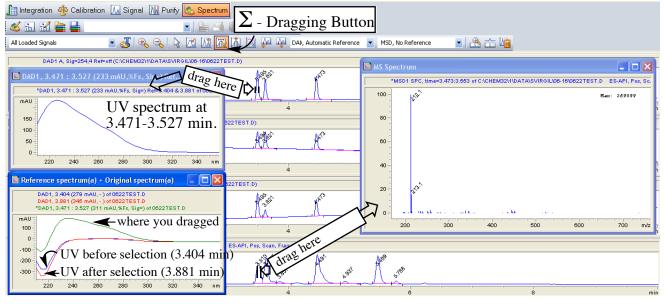
The "Spectrum" button allows one to view the Mass Spectrum or UV spectrum of a selected peak.

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		3.
Spectra may	Al Loaded Signals	nal A 🛛 📮
be selected		th = 254 nm
individually	2) DAD1 D, SIg=230,4 Ref=360,100 (C:)CHEM32(1)DATA(SYIRGIL)06-16(0622TEST.D)	<u>ui – 254 min</u> –
or all together	3) DAD1 G, Sig=280,4 Ref=360,100 (C:\CHEM32(1\DATA\SVIRGIL\06-16\0622TEST.D) 4) MSD1 TIC, MS File (C:\CHEM32(1\DATA\SVIRGIL\06-16\0622TEST.D)	545 STS
as shown.	0 2 4	6
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B 2 07-15	200	+ An er
± ⊆ 09-14 _		
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	🚳 INSTRUMENT 1 (offline): Data
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Delay sample runs until after Midnight	Overlay Signal
Condy sumple rand and one manight	Subtract Blank Run
Print report	Extract Ions
	Overlay Base Peak
	Subtract Background (BSB)
	Sabardee Baarigi Garia (BSB)

UHPLC-MS Data Analysis (continued)

Shown below, the dragging button delivers a spectrum for the region that the user drags the mouse over. The UV spectrum is created by subtracting the red and blue UV absorbances from the dragged region in order to compensate for solvent absorbance (the acetic acid used in the aqueous mobile phase absorbs in the 210 - 230 nm range).



Extract Ions – used to search for a mass or to evaluate the MS purity of a peak:

R INSTRUMENT 1 (offline): Data Analysis							
ile Method Sequence Graphics Integration Calibration Report Spectra Batch View Abort Help							
Load Signal Overlay Signal Subtract Blank Run	Extract lons: INSTRUMENT 1						
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Overlay Base Peak Subtract Background (BSB)	Extracted Ion Table		Sample Name Sample Info				
Snapshot	Enterions to be extracted. A single ion may be specified in column Ion1, or a range using Ion1 and Ion2.						
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Copy Delete			4 6				
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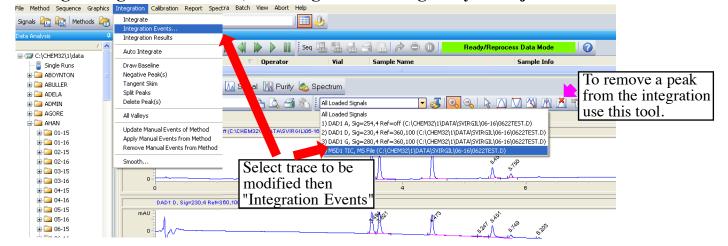
UHPLC-MS Data Analysis (continued)

Modifying Data Analysis Preferences – The first thing to know is how to get back to the default "LCMS ANALYSIS" Method since previous users often change settings.

🚟 INSTRUMENT 1 (offline): Da	ta Analysis		
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Extract Ions	Use current method 🔄 🕅	Name: Folders:	OK
Overlay Base Peak Subtract Background (BSB)	Overlay Date Time	LCMSANALYSIS.M c:\chem32\1\methods	Cancel
Snapshot	🛅 Integration 👋 Calji 📈 ion	CIR. PES100%, 650.M DEF_LC_PM.M LCMSANALYSIS M → chem32	
Import File Export File	🍝 🔝 🚮 🚞 🔽	LCMSARAETSIS M LCMSISHORT.M NEST04XM NEST04X 00-30.M	
Export MS Data to AIA Batch Convert MS Data to AIA	All Loaded Signals	NESTOA-40-95.M NESTOBX.M	
New	DAD Sig=254,4 Ref=	off Types: Drives:	
Load	Method	Method(".M)	Network
Save	Library		
Save As	Batch		

Methods for Integration:

- 1. Using the Integration button, a series of tools appear for manual integration.
- 2. Using "Integration Events", the integration settings may be adjusted:



Integration Events: showing how to raise the Area reject:

Integration ᢤ Calibration 📶 Signal 🛄	Purify 📥 Spectrum		UMENT 1 (offline): Data Analysis			
		File Met	File Method Sequence Graphics Integration Calibration Report Spectra			
🗓 🚯 🖌 Hit Green Chec		Signals	Signals 🦣 📷 Methods 👦 Integrate			
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UHPLC-MS Data Analysis (continued)

Printing Report: The "Specify Report" Window is used to select the format and destination of the printout. When re-loading the LCMSANALYSIS method, it is set to the printer. One can preview by selecting "Screen" or print to file formats such as pdf. When satisfied, Select **Report:Print Report**.

