0

Protocol: Exploring LCMS-MS data

Part I: Determining Exact Mass using ChemDraw

- 1. Navigate to the **PLNU virtual desktop** (https://view.pointloma.edu/).
 - Select the VMware Horizon HTML Access option.
 - Login with your PLNU credentials.
 - Select "Computer Lab Student."
- 2. Save the .cdx file onto the virtual desktop.
 - **From within the virtual desktop**, open a browser, and log into Canvas. From in Canvas on the virtual desktop, navigate to this page and open this link:

\$WIKI_REFERENCE\$/pages/chemdraw-files-for-week-9-lcms

(https://canvas.pointloma.edu/courses/56791/pages/chemdraw-files-for-week-9-lcms)

(https://canvas.pointloma.edu/courses/56791/pages/chemdraw-files-for-week-9-lcms)



- Download both .cdx files and note where they save (probably the Downloads folder).
- Note that you have to be in the virtual desktop window when downloading. If you try to do it from another tab in the browser on your computer, you'll be saving the file to your computer, instead of to the computer lab computer.
- 3. Open the file in ChemDraw.
 - From *within the virtual desktop,* Click on the Start Menu icon to open the start menu, and select ChemOffice 2019, followed by ChemDraw 19.1.

	😰 Point Lossa Nazarere Universi: 🗙 🛛 🔂 CHE2051 Week 1 - Google Do: 🗙 🕴 😳 Rotacut Exploring SCH5-M5 : 🛪 🙄 VWeare Harizon 🛛 🗴 🗖 Litetide presentation - Google: 🗴 🕇 🕂	- a ×
	€ → C O i vewpointoma.etu/pont/vet/dient/index/tmi#/deatop	🗅 🛪 🕲 i
	🗄 Apps 👩 Suggested lites 🍙 Speedtestaet by D. 🗧 Infectious Causes a. 👩 Vitaliourus Soluat. 🍪 Son 👔 Chrise Resources Sa. 🕲 New Tab. 🗖 Week & Problems	
	agyak Bin	
		4
0	Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion Image: Construction of the Statistic Deschartion of the Statistic Deschartion of the Statistic Deschartion Image: Construct Deschartion Image: Construction of the Statistic Deschartion of the Stati	
	a A H H 🧕 🖲 🗷 🖄	^ 10 00 1072820
	🖹 GMT20231007-1_mp4 A 🖹 GMT20231007-1_mp4 A 🗎 GMT20231007-125_0 A 🗟 GMT20231007-1_mp4 A 🗟 GMT20231007-1_mp4 A	Show all X
	🖽 🔎 Type here to search 🔹 🕒 🛤 🖪 🔞 🕫 🗷 🔨 🔺	48 958PM 🔍

- In the menu at the top of the ChemDraw screen, click File, then Open, then navigate to the file.
- 4. 'Ionize' the antibiotic structures. Recall that the mass spectrometer doesn't detect neutral molecules, but charged ions in this case (M-H)⁻ ions, also known as the conjugate base.
 - From the toolbar on the left side, click on the Chemical Symbols tool (the default is usually ⊕) and hold the button down to see the full set of options.



- Select the negative charge (\ominus) .
- Hover your cursor over the oxygen on one of the OHs on tetracycline, until the O is highlighted. Click once to impart a negative charge on this oxygen. (The hydrogen will disappear.)
- Repeat for one of the OH groups in each of the other antibiotics.
- 5. Check the masses you determined in your pre-lab using ChemDraw.
 - In the top menu, click View, then make sure Show Analysis Window is checked.
 - Using the lasso tool (top left in the toolbar), select one of the antibiotics.
 - The molecular formula, exact mass, molecular weight, etc. will appear in the Analysis Window.

	😰 Point Loma Nazarene Universit 🗴 📋 GHE3051 Week 1 - Google Dot 🗴 🔯 Protocol: Exploring LCMS-MS t 🗴 📮 VMware Horizon 🗴 📮 Unititled presentation - Google 🗴 🖡	÷	- 0	×
	← → C ①	☆	🗅 🛪 📧	:
	🔛 Apps 🚺 Suggested Sites 🍘 Speedtest.net by O 😌 Infectious Gauses o 💟 VitalSource: Educat 🤪 Jive 🚦 Online Resources fo 🔇 New Tab 🗖 Week 4 Problems			
	🕲 ChemDraw Professional - [Week 9 LCMS - antibiotics.cdx *] 👿 File Edit View Object Structure Text Curves Colors Search Add-ins Window Help		- 0	×
o	$\begin{array}{c c c c c c c c c c c c c c c c c c c $			^
	~ š. < ▷ □			× >
			へ 町 dw) ^{9:40 F}	PM
			10/7/2	020
	🖻 GMT20201007-1mp4 ^ 🖻 GMT20201007-1mp4 ^ 🗎 GMT20201007-175txt ^ 🖻 GMT20201007-1mp4 ^ 🖻 GMT20201007-1mp4 ^		Show all	×
	📲 🔎 Type here to search 🛛 O 🖽 🧮 🕅 🔯 🗷 🦉 🧮	^ <i>(</i> , (\$±40 PM ↓)) 10/7/2020 ■	3

- Adjust the number of Decimals to 4. Uncheck the boxes next to Mol. Wt., m/z:, and Elem.
 Anal. Then click Paste.
- Repeat with the other antibiotics. How do these values compare with the ones you calculated?
- You can minimize the virtual desktop for now, but don't close it. (You'll need to use ChemDraw again in step 8 of Part II.)

Part II: Exploring the LCMS of a mixture of antibiotics

1. Visit gnps.ucsd.edu (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp) and log into your



usi=mzspec%3AMSV000086079%3Aantibiotics_1ug_ml_neg_20200902112656%3Ascan%3A1000&usi 2=&xicmz=&xic_tolerance=0.5&xic_rt_window=&xic_norm=False&xic_file_grouping=FILE&show_ms 2_markers=True&ms2_identifier=None) 3. Open the antibiotic mixture datafile. Under the **File Selection** tab, in the **GNPS USI** field, delete the default file path and paste the following:

mzspec:MSV000086079:antibiotics_1ug_ml_neg_20200902112656:scan:1000, then click Link to these plots.

	🕐 Topic: Week 1 Discussion: Int: 🗙 🙆 My Drive - Google Drive 🛛 🗙 🚍 LCMS activity - Google Docs	x 🔤 GNPS - LCMS Browser x 🕿 GNPS - Analyze, Connect, and x + - 🗗 X
	 C ① ① ● gnps-lcms.ucsd.edu Apps D Suggested Sites ⑦ Speedtest.net by O ② Infectious Causes o ♥ VitalSource: Educat 	🔅 🗈 🔅 🕃 😪
	GNPS GNPS LCMS Dashboard - Version 0.8	í
	Data Selection	
	File Selection	XIC Options
~	GNPS USI mzspec:MSV000086079:antibiotics_1ug_ml_neg_20200902112656:scan: 1000	XIC m/z Enter m/z to XIC
0	GNPS USI2 Enter GNPS File USI	XIC Tolerance 0.5 XIC Normalization • XIC File Grouping By File*
	Enter USI Above or Drag and Drop your own file or Select Files	XIC Retention Time Window Enter RT Window (e.g. 1-2)
	Link to these plots	MS2 Identifier Enter Spectrum Ident Show MS2 Markers
		SVG Sty simple_white
	https://gnps-tcms.ucsd.edu//usi=mzspec%.sAMbYUUUUUb//9%3Aantbiotics_1ug_ml_neg_20200902112656%3Ascan%3A1	UUUUUuusid=Boocmiz=Boocmiz=Boocmid=Boocmid=BioleBoocmid BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=BioleBoocmid=B
	f 🔎 Type here to search O 🖽 🗖 🧃 🥊	x 🗄 📴 🔹 ^ 🧟 49 855 PM

4. Save an image of the TIC plot. Scroll to the bottom of the page (left side). If you hover your cursor over the TIC plot, a set of icons will appear directly above, including one that looks like a camera. Click on this icon to download an image (as an SVG file) of your TIC to include with your postlab report.

C	GNPS GNPS LON	rtS Dashboard - Version 0.8	
	TIC Plot	Q 9, ÷ □ □ × ≠ Download plat	T = = Contributors
	350M - 200M - 250M - 200M - 월 150M -		Mingsun Wang PhD - UC San Diego Wout Bitteenieux PhD - UC San Diego Benjamin Pulman - UC San Diego Daniel Petras PhD - UC San Diego Waress Phelan PhD - CU Anchutz Tristan de Rond PhD - UC San Diego Alan Jarmusch PhD - UC San Diego
	100M - 50M -	Red Lancesti	DEBUG Panel
	02	4 6 8 rt	tip://massive.ucsd.edu/MSV000086079/peak/antibiotics_tug_ml_neg_202009021126 56/maML Using UBL USI

- 5. Now let's explore the **TIC plot.** The **TIC** or **Total Ion Chromatogram** is a plot of the total number of ions (of all masses) hitting the detector at a given time. The y-axis shows the number of ions, while the x-axis shows the **retention time (rt)** in minutes. When a molecule such as an antibiotic is coming off the chromatography column, the total number of ions goes up, and then down as the compound tapers off. In this plot, each peak corresponds to a molecule.
 - Hover your cursor over a peak, such as the tallest one in the spectrum, between 2 and 4 minutes. When you are near a peak, a little box will pop up with the exact retention time (rt) and total ion counts (tic) for that peak.



- Click on the peak at ~2.8 min. Wait a few seconds, while the mass spectrum (MS1) loads to the right of the TIC plot. The MS1 (also just known as the mass spectrum) shows each of the masses detected at a given time (in this case 2.8 minutes). The x-axis shows the mass of each ion (m/z), while the y-axis shows the intensity (number of ions hitting the detector) corresponding to each mass. A typical mass spectrometry dataset consists of *hundreds* or *thousands* of mass spectra like this one - one corresponding to each datapoint in the TIC plot!
- Now scroll right and hover your cursor near the top of the large peak in the MS1. Another little box will appear, showing the mass of that peak. This is the molecular ion for the antibiotic that came off at 2.8 minutes. Based on the calculations you did for your pre-lab, which antibiotic is this?



- 6. Find each antibiotic in the TIC plot. Repeat step 7 for the peaks at 2.1 (small peak), 3.3 (medium-sized peak) and 4.9 minutes. Which antibiotic does each correspond to? Download an image of one of the MS1 spectra to include with your postlab report.
- 7. Let's explore something called the **XIC**. In this example, our total ion chromatogram (TIC) is quite 'clean'. There were only a few purified antibiotics in this sample, and these show up as distinct peaks in the chromatogram. In other cases such as when we are looking at natural product

extracts - the TIC may be packed with peaks, most of which we are not interested in. In that case, we can quickly pull out peaks of interest using something called an **extracted ion chromatogram** (or **XIC**).

Scroll back to the top of the page. Under XIC Options, type the exact mass of the conjugate base of penicillin (the one given to you in your prelab): 333.0915. Scroll down and wait a few seconds for the XIC Plot to appear under the Details Panel. The XIC Plot will show *only* the ions that have an exact mass of 333.0915. What does the XIC Plot look like?

Are to be presented by the presented by the presented by the presented by the base base of the base base base base base base base bas) Topic: Week 1 I → C 企	Discussion: Intr 🗙 📔	My Drive - Goo ucsd.edu/?usi=m:	izspec%3AMS\	V000086079%3	3Aantibiotics_1ug	_ml_neg_202	00902112656	%3Ascan%3A1	000&usi2=&xic	mz=8xic_tol	erance=0.5&	xic_rt	☆	• *	К
Data Selection File Selection	GNPS	GNPS LCM:	eedtest.net by O Dashboard - V	8 Infectious	s Causes o	VitalSource: Edu	cat 🤮 Jiw	e 🚹 Online	Resources fo	🕑 New Tab 🕻	Week 4 Prol	blems				
File Selection XIC Options GNPS US2 Enter GNV5000660793antibiotics, lug, mL, reg, 2020092112555s.scm NC m/2 333.015 GNPS US2 Enter GNV5 Tile USI NC Tolerance S NC Normalization Brite File MS2 US2 Enter GNV5 Tile USI NC Tolerance S NC Normalization Brite File MS2 Options NC Tolerance S NC Normalization Brite File MS2 Options NS US2 Store of the plots Store of the plots Store of the plots VM conserved pull_off Th C2 Jag Th C1 Jag Membran paper plot Membran paper plot Membran Paper plot Paper here to a seach My Dow-Googe Date Yes Holdowere face Moders- Adapted Store Cole and Store T, Windowere face Membran Paper plot	Data Selec	ction														
Image CMS USI msge CMS USI Inter GMPS File USI Image CMS USI Enter GMPS File USI Image CMS USI Image CMS USI Image CMS USI Image CMS USI Enter GMPS File USI Image CMS USI USI USI USI USI USI USI USI USI US	File Selection			хі	C Optior	IS										
KIC Tolerance 0.5 XiC Normalization * XiC File Grouping By File* By File* Enter USI Above or Drag and Drop your own file or Select Files KiC Retention Time Window Enter RT Window (e.g. 1-2) MS2 Options MS2 Identifier MS2 Identifier Stor Sty sinple_white Prove the to search Wind creased pulsation * KiC Retention Time Window File* Not creased pulsation * KiC Retention Time Window File* Not creased pulsation * KiC Retention Time Window File* Not creased pulsation * KiC Retention Time Window File* Not creased pulsation * KiC Retention Time Window File* Not creased pulsation * KiC Retention Time Window File* Not creased pulsation File* Stor Sty Sinple_white Stor Sty Sinple_white Store Store Sty Sinple_white Store Store<	GNPS USI	mzspec:MSV0 1000	00086079:antib	piotics_1ug_n	ml_neg_2020	0902112656:sca	an:	KIC m/z	333.0915							
Enter USI Above or Drag and Drop your own file or Select Files Link to these plots Link to these plots Not crossed pu_pet Int to table plot Not crossed pu_pet Not crossed plot plot plot	GNPS USI2	Enter GNPS (ile USI					KIC Tolerand	ce 0.5	XIC Normal	ization	• XIC	∶File Gr y File≖	ouping		
Either GSA Addree of Unlag and Unlag your own me of select rinks MS2 Options MS2 Address MS2 Markers SVS SVS SVy Simple, white For Unlag and Unlag your own me of select rinks MS2 Address SVS SVS SVS SVy Simple, white For Unlag and Unlag your own me of select rinks MS2 Address SVS		Enter LISI Above	or Drog and Dr		file or Color	t Filos	<u>م</u>	KIC Retentio	on Time Wind	ow Enter R	T Window (e.g. 1-2)				
Inik to these plots MS2 Identifier MS1:1714 Show MS2 Markers Show MS2 Markers Store <td></td> <td>Enter OSI ADOVE</td> <td></td> <td>op your own</td> <td></td> <td></td> <td>. м</td> <td>S2 Optio</td> <td>ns</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>		Enter OSI ADOVE		op your own			. м	S2 Optio	ns							
SVG Sty simple_white NVT crossword pu_pet It Cc 1jpg It marbasin paper,pdf It marbasin Bapm Several P Type here to search It Cc 1jpg It marbasin paper,pdf It marbasin Bapm Several P Type here to search It Cc 1jpg It marbasin paper,pdf It marbasin Bapm Several P Type here to search It Cc 1jpg It marbasin paper,pdf It marbasin Bapm Several P Type here to Search My Dhe-Google Dhe It Cl 1jpg It Marbasin Paper,pdf It marbasin Bapm Several P Type here to Search My Dhe-Google Dhe It Cl 1jpg It Marbasin Paper,pdf It marb			Link to th	nese plots			N	VIS2 Identifi	ier MS1:17	14	Show N	1S2 Markers	s •)•	
NYT crossword pu_pdf TLC 1,pg Introduction TLC 1,pg Introduction Introduction <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td>Re</td><td>endering</td><td>Options</td><td></td><td>Stv</td><td>simple w</td><td>hiter</td><td></td><td></td><td></td></td<>							Re	endering	Options		Stv	simple w	hiter			
MYT crossword pu_pdf ^ P Type here to search																
P Type here to search P P Type here to search P P P Type here to search P P P P P P P P P P P P P P P P P P P								_								
3 Topic Week 11 Discussion: Int: x ▲ My Drive - Geogle Drive x ▲ LONS activity - Geogle Doc x x ▲ GNRS - LONS Browser x ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 11 Discussion: Int: x ▲ My Drive - Geogle Drive x ▲ GNRS - LONS Browser x ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 11 Discussion: Int: x ▲ My Drive - Geogle Drive x ▲ GNRS - LONS Browser x ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 11 Discussion: Int: x ▲ My Drive - Geogle Drive X ▲ GNRS - LONS Browser x ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive X ▲ GNRS - LONS Browser X ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive X ▲ GNRS - LONS Browser X ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive X ▲ GNRS - LONS Browser X ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive X ▲ GNRS - LONS Browser X ▲ CNRS - Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive X ▲ GNRS - LONS Browser X ▲ CRASS - Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive A Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Geogle Drive A Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Store A Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Store A Analyze, Connect and x + □ 3 Topic Week 12 Discussion: Int: x ▲ My Drive - Store A Analyze, Connect and x + □ 3 Topic Week 12 Discussion: A Analyze, Connect and x + □ 3 Topic Week 12 Discussion: X →	NYT crosswor	rd pupdf \land	TLC 2.jpg	,	~ 📄 тис	C 1.jpg	~ @	B maribasin	paper.pdf	^ 🖹 ma	ribasin B.png	^			Sh	ow all
Y Construction of the second state of the second state of the second state and the se	NYT crosswor	rd pupdf ^	TLC 2.jpg	0	~ 🖻 тыс Ці 🗖	C 1.jpg	~ (🖻 maribasin	paper.pdf	^	ribasin B.png	^		^ <i>(i</i> . d	9:04)) 9:04 10/5/	PM (2020
ONE Deshboard - Version 0.8 Data Exploration Details Panel Image: state of the state o	NYT crosswor	rd pupdf ^	 TLC 2.jpg My Drive - Goo 	O ogle Drive	^ ■ τια Ħ t	C 1.jpg	∧ (x∃ F Docs x [Maribasin	paper.pdf 15 Browser	 ma x < GNF 	ribasin B.png 'S - Analyze, Co	nnect, and X	+	^ <i>(i</i> , d	Sh ≫ 9:04 >>) 10/5/ 	PM (2020
Data Exploration Details Panel Details Panel Storation Details Panel Details Panel Storation Details Panel Storation Details Panel Storation S	 NYT crosswor <i>P</i> Type h <i>Topic:</i> Week 1 1 <i>→</i> C ① <i>Apps</i>	rd pupdf ^	TLC 2.jpg My Drive - Goo ucsd.edu/?usi=m: eedtest.net by O	ogle Drive	 ▲ ▲ ★ ↓ ■ ■	S activity - Google I BAantibiotics_1ug VitalSource: Edu	∧ (X II F Docs X II _ml_neg_202i _at ⊗ Jive	maribasin maribasin GNPS - LCN 00902112656 e T Online	paper.pdf 15 Browser 5%3Ascan%3A1 Resources fo	 ma x < GNF 0008cusi2=8cxic New Tab 	ribasin B.png ^{IS} - Analyze, Co :mz=&txic_tol- Week 4 Prol	nnect, and X erance=0.5& blems	+ +	^ <i>(i</i> , ⊄	Sh 9:04 10/5/ —	pw all PM 2020 CP
$\begin{array}{c c} \text{Let up for the form } \\ \hline \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	NYT crosswor	rd pupdf ^ here to search Discussion: Int: × i @ gnps-Icms. gested Sites (?) Sp	TLC 2.jpg My Drive - Goo ucsd.edu/?usi=m. eedtest.net by O Dashboard - V	ogle Drive Izspec%3AMS\ © Infectious	▲ ILC ↓ ↓ × ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	C 1.jpg V 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	^ ₽ X∃ ₽ Docs X ∎ _ml_neg_2021 :at ⊗ Jive	maribasin	paper.pdf IS Browser i%3Ascan%3A1 Resources fo	 ▲ ma ★ ● GNF ★ ● GNF ★ O008cusi2=8cxic ♦ New Tab 	ribasin B.png ^{IS} - Analyze, Co :mz=&xic_tol Week 4 Prol	rinnect, and x erance=0.5&z blems	+ xic_rt	^ <i>⋒</i> ⊄ ☆	Sh 9:04 10/5/ - 3	ow all 2020
Abundance 1.e ³ 1.e	NYT crosswor P Type h Topic: Week 1 1 → C Δ Apps D Sugg Data Explo	rd pupdf ^ here to search Discussion: Int: × h @ gnps-Icms. gested Sites @ Sp GNPS LCMS	TLC 2.jpg My Drive - Gos ucsd.edu/?usi=mu eedtest.net by O Dashboard - V	ogle Drive Izspec%3AMSV S Infectious Version 0.8	 ▲ TLC ➡ TLC ➡ ■ LCM × ➡ LCM × ➡ LCM s Causes o ▲ 	C 1.jpg S activity - Google I 3Aantibiotics_1ug V VitalSource: Edu	∧ (X] P Docs X [_ml_neg_2021 rat (3) Jive	Maribasin	paper.pdf IS Browser i%3Ascan%3A1 Resources fo	∧ ■ ma ×	ribasin B.png ^{IS} - Analyze, Co :mz=&xic_tol Week 4 Prol	nnect, and X erance=0.5&z blems	+	^ <i>í</i> d \$	Sh) 9:04 10/5/ -	PM (2020 (D K
XIC Plot - Single File XIC Plot - Single File XIC Plot - Single File XIC Plot - Single File XIC Plot - Single File MS2 Acquisitions MS2 Acquisitions	 NYT crosswor P Type h Topic: Week 111 P C Q Apps Sugg GNPS Data Explo 	rd pupdf ^ here to search Discussion: Int: × I @ gnps-Icms igested Sites (?) Sp GNPS LCMS pration	TLC 2.jpg My Drive - Goo ucsd.edu/?usi=m. ieedtest.net by O i Dashboard - V	ogle Drive Izspec%3AMSV & Infectious Version 0.8	▲ TLC ↓ ↓ × ↓ ↓ ↓ LCM V000086079%: s Causes o ↓	S 1jpg S activity - Google I 3Aantibiotics_1ug VitalSource: Edu	∧ x∃ ml_neg_2021 cat (?) Jiw	maribasin GNPS - LCN GNP GNP GNP GNP GNP GNP GNP GN	paper.pdf IS Browser 5%3Ascan%3A1 Resources fo Panel	∧ na ma × GNF 0008tusi2=8xio	ribasin B.png IS - Analyze, Co Imz=&xic_tol- Week 4 Prol	rancet, and X erance=0.5& blems	+	^ <i>ଲ</i> ⊄ ☆	Sh 9:04 10/5/ - 3	ow all
Abundance 1.e9 1.e7 1.e6 1.e5	 NYT crosswor P Type h Topic: Week 1 1 P C G Apps Sugg GNPS Data Explo 	rd pupdf ^ here to search Discussion: Int: × t	My Drive - Goo ucsd.edu/?usi=m: reedtest.net by O	ogle Drive zzspec%3AMSV & Infectious Version 0.8	 ▲ TLC ★ ➡ ➡ ➡ ➡ LCM × ➡ ➡ ➡ ➡ LCM v000086079%3 s Causes o ▲ 	C 1.jpg V J Q Q IS activity - Google I 3Aantibiotics_1ug V VitalSource: Edu	n (i x] P ml_neg_2021 cat (i) Jiw	maribasin maribasin GNPS - LCN GNPS - LCN O00902112656 e ① Online Details F	paper.pdf IS Browser 5%3Ascan%3A1 Resources fo Panel	▲ ma × ● GNF 000&cusi2=8xxic ③ New Tab	ribasin B.png IS - Analyze, Co Imz = &xxic_tol	innect, and X erance=0.5&	+ xic_rt	^ <i>(</i> , ⊄ ☆	Sh 9:04 10/5/ - 3	PM (2020)
1 col 1 col 1 col 40H watable 1 200 1 col 1 col 30H M 1 col 1 col 30H M MS2 Acquisitions 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col 1 col <td< td=""><td> NVT crosswort P Type In Topic Week → C Δ Apps Is Sugg GNPS Data Explo </td><td>nd pupdf ^ here to search Discussion: Int: X i @ gnps-Icms gested Sites (?) Sp GNPS LCMS oration</td><td>TLC 2.jpg My Drive - Goo ucsd.edu/?usi=m. reedtest.net by 0 Dashboard - V</td><td>ogle Drive Zzspec%3AMSV € Infectious /ersion 0.8</td><td>▲ I TLC ↓ ↓ × ↓ ↓ LCM × ↓ ↓ LCM × 000086079%: s Causes o ↓</td><td>C 1.jpg</td><td>∧ (i ×] (i ml_neg_202i cat (i) Jiw</td><td>maribasin GNPS - LCN GOUDDO2112656 Tonline Details F XIC F</td><td>paper.pdf IS Browser 1%3Ascan%3A1 Resources fo Panel</td><td> A ma A ma A CONF <</td><td>ribasin B.png IS - Analyze, Co Imz=8txic_tol Week 4 Prol</td><td>nnect, and X erance=0.5& blems</td><td> + xic_rt</td><td>∧ <i>m</i> ⊲ ☆</td><td>Sh 9:04 10/5/ - 3</td><td>ow all 2020 ([™] [™] [™]</td></td<>	 NVT crosswort P Type In Topic Week → C Δ Apps Is Sugg GNPS Data Explo 	nd pupdf ^ here to search Discussion: Int: X i @ gnps-Icms gested Sites (?) Sp GNPS LCMS oration	TLC 2.jpg My Drive - Goo ucsd.edu/?usi=m. reedtest.net by 0 Dashboard - V	ogle Drive Zzspec%3AMSV € Infectious /ersion 0.8	▲ I TLC ↓ ↓ × ↓ ↓ LCM × ↓ ↓ LCM × 000086079%: s Causes o ↓	C 1.jpg	∧ (i ×] (i ml_neg_202i cat (i) Jiw	maribasin GNPS - LCN GOUDDO2112656 Tonline Details F XIC F	paper.pdf IS Browser 1%3Ascan%3A1 Resources fo Panel	 A ma A ma A CONF <	ribasin B.png IS - Analyze, Co Imz=8txic_tol Week 4 Prol	nnect, and X erance=0.5& blems	+ xic_rt	∧ <i>m</i> ⊲ ☆	Sh 9:04 10/5/ - 3	ow all 2020 ([™] [™] [™]
1200 1000 1.e8 1.e8 1.e8 1.e8 1.e8 1.e7 1.e5	NYT crosswor P Type I Dropic Week 11 → C Ω Apps © Sugg Data Explo	rd pupdf ^ here to search Discussion: Int: X i @ gnps-Icms gested Sites @ S GNPS LCMS oration	TIC 2 Jpg My Drive - Gou ucsd.edu/?usi=m. eestest.net by 0 Dashboard - V	ogle Drive Zzspec/63AMSU & Infectiouz Version 0.8	▲ ■ TLC ■ TLC ■ ■ TLC × ■ LCM × ■ LCM <td>2 Jjpg S activity - Google I SAantibiotics, Tug VitalSource: Edu Abundance</td> <td>∧ (i ×) r mi_neg_2021 cat (i) Jiw</td> <td>maribasin GNPS - LCM GNPS - LCM GNPS - LCM D00902112650 e Online Details F XIC F</td> <td>paper.pdf IS Browser 1%3Ascan%3A1 Resources fo Panel</td> <td>x Contraction of the second se</td> <td>ribasin B.png IS - Analyze, Co Imz=&xxic_tol: Week 4 Prol</td> <td>nnect, and X erance=0.5& blems</td> <td> + xic_rt</td> <td>^ <u>∭</u> ⊄ ☆</td> <td>Sh 9:044 - - - - - - - - - - - - -</td> <td>iii</td>	2 Jjpg S activity - Google I SAantibiotics, Tug VitalSource: Edu Abundance	∧ (i ×) r mi_neg_2021 cat (i) Jiw	maribasin GNPS - LCM GNPS - LCM GNPS - LCM D00902112650 e Online Details F XIC F	paper.pdf IS Browser 1%3Ascan%3A1 Resources fo Panel	x Contraction of the second se	ribasin B.png IS - Analyze, Co Imz=&xxic_tol: Week 4 Prol	nnect, and X erance=0.5& blems	+ xic_rt	^ <u>∭</u> ⊄ ☆	Sh 9:044 - - - - - - - - - - - - -	iii
E 800 1.e7 1.e6 1.e5 NYT crossword pu_pdf ^ It C2 jpg ^ It C1	NYT crosswor P Type I Topic Week 11 P C C Apps 2 Sugg CONPC Data Explo	rd pupdf ^ here to search Discussion: Int: × t @ gnps-Icms grasted Sites (?) \$ S GNPS LCM: oration	TIC 2 Jpg My Drive - Go ucsd.edu/?usi=m eestest.net by 0 Dashboard - V	ogle Drive zzspec/63AMSV & Infectiouz Version 0.8	▲ ■ TLC ↓ ■ TLC × ■ LCM V0000086079%: s Causes o ■	E 1.jpg S activity - Google I SAantibiotics_ Lug VitalSource Edu Abundance 1.e9	∧ (i x∃ f ml_neg_202i at (i) Jiw	maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin	paper.pdf IS Browser 19%3Ascan%3A1 Panel Pot - Single	x Conformation of the second s	ribasin B.png 'S - Analyze, Co 'T - Analyze, Co 'T - Analyze, Co 'Week 4 Prol	A series of the series of	+ + ≥ ≈ + varia	☆ / / / / / / / / / / / / / / / / / / /	Sh) 9:04 10/5/ - - 3.0915	all PM [C ³ K K
E 800 600 1.e5	NYT crosswore	rd pupdf ^ here to search Discussion: Int: × t @ gnps-Icms greated Sites () s GNPS LCM: oration	TIC 2 Jpg My Drive - Go uccd edu/?Lusiem eentestinet by O Dashboard - V	ogle Drive ZZSPEC%3AMS\ € Infectiouz /ersion 0.8	 ▲ I TLC ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	E 1.jpg S activity - Google B Adantibiotics, Tug VitalSource: Edu Abundance 1.e9 1.e9	A E	maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribasin maribas	paper.pdf 15 Browser 163 Ascan%3A1 184 Ascan%3A1 Resources fo	∧ N ma × ⊂ GNF 0008cusi2=8csic ⊗ New Tab C File	ribasin B.png IS - Analyze, Co Imz=8oxic_tol- Week 4 Prol	nnnect, and x erance=0.5& blems ·	+ + varia varia	∧ ^(k) d ★ <p< td=""><td>Sh 9:040 - - - - - - - - - - - - -</td><td>PM (2020 (C C C C C C C C C C C C C</td></p<>	Sh 9:040 - - - - - - - - - - - - -	PM (2020 (C C C C C C C C C C C C C
E 800 1.e6 1.e6 1.e6 1.e5 1.e5 rt NYT crossword pu…pdf ^ E TLC 1/pg ^ E maribasin Baper, pdf ^ E Show all	NYT crosswor P Type t Topic: Week 1 [→ C ① ∴ Apps D Sugr → C ① Sugr → C ① Sugr → C ① ↓ → ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓ → C ① ↓	rd pupdf ^ here to search Discussion: Int: × t @ gnps-Icms gested Sites (?) SI S GNPS LCM: oration	TIC 2 jpg My Drive - Go used dedu/Tusin m eedtestnet by O Dashboard - V	vogele Drive Zzspec%SAMSV ≷ Infectious /ersion 0.8	 ▲ I TLC ➡ TLC ➡ ECM × ■ LCM × ■ LCM × □ LCM <li< td=""><td>E 1.jpg S activity - Google 1 Abundance Abundance Le9 Le9 Le9 Le9</td><td>A (X) P ml_neg_202 cat (3) Jive</td><td>maribasin 교 교 교 교 교 교 교 교 교 교 교 교 교 Details F XIC F 40M 고 고 실 고</td><td>paper.pdf 65 Browser 9%3Ascan%3A1 Resources fo Plot - Single</td><td>▲ ma × Constant of the second sec</td><td>ribasin B.png IS - Analyze, Co Imz=&xic_tol Week 4 Prol</td><td>nnect, and X erance=0.5& blems</td><td>+ + = × + varia</td><td>ヘ <u> </u></td><td>Sh 9:040 - - 3.0915 3.0915</td><td>mini mini</td></li<>	E 1.jpg S activity - Google 1 Abundance Abundance Le9 Le9 Le9 Le9	A (X) P ml_neg_202 cat (3) Jive	maribasin 교 교 교 교 교 교 교 교 교 교 교 교 교 Details F XIC F 40M 고 고 실 고	paper.pdf 65 Browser 9%3Ascan%3A1 Resources fo Plot - Single	▲ ma × Constant of the second sec	ribasin B.png IS - Analyze, Co Imz=&xic_tol Week 4 Prol	nnect, and X erance=0.5& blems	+ + = × + varia	ヘ <u> </u>	Sh 9:040 - - 3.0915 3.0915	mini mini
600 2 4 6 8 rt	NYT crosswor P Type I Drpic Week 11 → C ① Appr D Sugg Data Explo	rd pupdf ^ here to search Discussion: Int: X	 TLC 2.jpg My Drive - Go uscal.edu/?usi=m. eedtest.net by 0 Dashboard - V 	ogle Drive zzspec%3AMSV & Infectious Version 0.8	 ▲ TLC ★ ■ TLC ★ ■ LCM × ■ LCM <	C 1 jpg	A E	maribasin ۲ ۲	paper.pdf 45 Browser 1963 Ascan%3A1 Resources fo Panel Plot - Single	∧ ■ ma × ● GNF × ●	ribasin B.png IS - Analyze, Co Imz=8cxic_tol Week 4 Prol	nnect, and X erance=0.5& blems	+ bic_rt ■ ≫ (varia	☆ / / / / / / / / / / / / / / / / / / /	Sh 3:04	all PM CD CD CD CD CD CD CD CD CD CD
600 Image: state s	NYT crosswor P Type I P Type I Topic Week II P Type I Supprovement Apps I Supprovement Idop Idop <td>rd pupdf ^ here to search Discussion: Int: X i i gnps-Icms ground Sites (?) Sr GNPS LCM: oration</td> <td>TLC 2 Jpg My Drive - Go ucsd.edu/?usi=m eedtest.net by 0 Dashboard - \</td> <td>ogle Drive zzspec%3AMSV Version 0.8</td> <td>A ■ TLC X ■ LCM X ■ LCM X 000066079%3 s Causes a ■</td> <td>Abundance 1.e9 1.e7 1.e6</td> <td>A B Abos X B ml_neg_2021 cat S Jiw</td> <td>maribasin ه (۲۹۲) ۱ (۲۹۶)</td> <td>paper.pdf 45 Browser 7963Ascan963A1 7963Ascan963A1 Panel 100 - Single</td> <td>x € GNF x € GNF S New Tab C</td> <td>ribasin B.png IS - Analyze, Co Immz=&xic, tol Week 4 Prol</td> <td>nnect and X erance=0.5& blems</td> <td> + xic_rt varia x</td> <td>A CALL AND AND AND AND AND AND AND AND AND AND</td> <td>Sh 9:04 10/5/ -</td> <td>In the second se</td>	rd pupdf ^ here to search Discussion: Int: X i i gnps-Icms ground Sites (?) Sr GNPS LCM: oration	TLC 2 Jpg My Drive - Go ucsd.edu/?usi=m eedtest.net by 0 Dashboard - \	ogle Drive zzspec%3AMSV Version 0.8	A ■ TLC X ■ LCM X ■ LCM X 000066079%3 s Causes a ■	Abundance 1.e9 1.e7 1.e6	A B Abos X B ml_neg_2021 cat S Jiw	maribasin ه (۲۹۲) ۱ (۲۹۶)	paper.pdf 45 Browser 7963Ascan963A1 7963Ascan963A1 Panel 100 - Single	x € GNF x € GNF S New Tab C	ribasin B.png IS - Analyze, Co Immz=&xic, tol Week 4 Prol	nnect and X erance=0.5& blems	+ xic_rt varia x	A CALL AND	Sh 9:04 10/5/ -	In the second se
NVT crossword pu_pdf ^ TLC 2.jpg ^ TLC 1.jpg ^ maribasin paper.pdf ^ maribasin 8.png ^ Show all	NYT crosswor P Type I P Type I P Type I Supple Vector Appr I Supple Vector Data Explo 1400 1200 1200 E 800	rd pupdf ^ here to search Discussion: Int: × i i gnps-Icms ground Sites () si GNPS LCM: oration	TIC 2 Jpg My Drive - Go ucsd.edu/?usi=m eestest.net by 0 Dashboard - \	ogle Drive zzspec%3AMSV ≷ Infectious /ersion 0.8	A ■ TLC → ■ LCM × ■ LCM × 000086079%3 s Causes o ■	Abundance Abundance 1.e9 1.e6	A B	maribasin ۲	paper.pdf IS Browser ISS Ascan%3A1 Panel Plot - Single	A ■ ma X ● GNF X ●	ribasin B.png IS - Analyze, Co Imz=&xcic_tol- Week 4 Prol @ @ ++ @	nnect, and X erance=0.5& blems	+ oxic_rt varia x	▲ / / ペ ペ ペ	Sh 9:04 10/5/ -	ow all PM 2020 (0 0 0 0 0 0 0 0 0 0 0 0 0
NYT crossword pupdf ^ TLC 2.jpg ^ TLC 1.jpg ^ manibasin paper.pdf ^ manibasin 8.png ^ Show all	NYT crosswor P Type I P Type I Topic Week II P Type I Support Support Component Ideo	rd pupdf ^ here to search Discussion: Int: × i @ gnps-tcms greated Sites () s S GNPS LCM: oration	TIC 2 Jpg My Drive - Go uesd.edu/?usi=m eesdtett.net by 0 Dashboard - V	ogle Drive zzspec%SAMSU € Infectiouz Version 0.8	A ■ TLC	Abundance 1.e9 2. VralSource: Edu 1.e8 1.e7 1.e6 1.e5	A Construction of the second s	maribasin	paper.pdf IS Browser IS Brow	A B ma X CAPE ONE CAPE OF CA	ribasin B.png IS - Analyze, Co Immz=&xic_tol Week 4 Prol © Q + 100 5	nnect, and X erance=0.5& blems	+ oric_rt varia x	A ⁽ _k d) ★ ^(k)	Sh 9:044	wall PM C2 C2 C2 C3 C4 C4 C4 C4 C4 C4 C4 C4 C4 C4
	 NYT crosswork P Type Ib → C ① App: D Sugo B Sugo Data Explo 1400 1400 1000 800 600 	rd pupdf ^ here to search Discussion int: × P @ gnps-Icms S GNPS LCM: oration	TIC 2 Jpg My Drive - Go uccd edu/Tusiem eeeftetinet by O Dashboard - V	vogele Drive Zzspec%SAMSV € Infectioux /ersion 0.8	A ■ TLC ■ ■ × ■ LCM ■ × ■<	Abundance Abundance 1.e8 Aantbiotiss Tug VitalSource Edu 1.e9 1.e8 1.e6 1.e5	A CONTRACTOR	maribasin ۲ ۲	paper.pdf 45 Browser 1953 Ascan 953 A1 1963 Ascan 953 An 1963 An 1973 An	∧ ■ ma × ⊂ GNF 0008cusi2=8oic ⓒ New Tab C File 4 rt	ribasin B.png (S - Analyze, Co mmz=8xic_tol Week 4 Prol	nnect, and X erance=0.5& blems	varia	A C A A	Sh 9:04 0/5/ - - - - - - - - - - - - - - - - - -	ew all

- In the XIC Options field, add a semicolon after the mass of penicillin, and add the other masses you calculated in your prelab for each of the antibiotics, separated by semicolons. How does this plot compare to the TIC plot?
- Download an image of the XIC plot of the four antibiotics to include with your postlab report.
- 8. Now let's explore the tandem mass spectrum (MS2 spectrum).
 - At the top right of the page, next to MS2 Options, check that the Show MS2 Markers toggle switch is toggled to the right ('on'). If not, press on the toggle switch once. (When Show MS2

0

Markers is 'on', you should see a series of tiny blue Xs in the 2D plot under **Data**

xploration.)	
)Topic: Week 1 Discussion: Int: 🗙 📔 🤷 My Drive - Google Drive 🛛 🗙 📔 🖬 LCMS activity - Google	Docx 🗴 🔝 GNPS - LCMS Browser 🗴 🗢 GNPS - Analyse, Connect, and 🗴 + 🦳 🗗
→ C O is graps-konsuesdedu/lusi=mespec%3AM5V000086079%3Aantbiotics, to Appr Suggested lines (A Sprediester Up 0 B) Infections Cause a Ø Vitaliaures da	g. nel., neg., 20201902 († 12556 NJ. Accarlis J.A. 1000 du siz-edu i c., talestance = 8.5 koi c. nt
GNPS GNPS LCMS Dashboard - Version 0.8	
Data Selection	
File Selection	XIC Options
GNPS USI 825pecMSV000086079:antibiotics,1ug,ml,neg,2020090211265630 1000	xxm: xxC myz 333.0915
GNPS USIZ Enter GNPS File USI	XIC Tolerance 0.5 XIC Normalization • 200 FRe Grouping By File*
	30C Retention Time Window Enter RT Window (e.g. 1-2)
Enter USI Above or Drag and Drop your own nie or select Hies	MS2 Options
Link to these plots	MS2 Identifier MS1:1714 Show MS2 Markers • • •
	Rendering Options
	SVG v Sty simple_white
NYT conservent pall-path 🔺 🖻 m.c. signs	n 🔒 maribasin paper pdf n 🔒 maribasin tupog n Show all
	98.04

Scroll down to the 2D plot under the Data Exploration heading. This somewhat overwhelming 2D plot shows the combined information from all the MS1 spectra in this dataset! The x-axis shows retention time, while the y-axis shows mass-to-charge ratio. Each vertical slice in the plot corresponds to the MS1 spectrum at that retention time. If you look at the retention times corresponding to our antibiotics, you can see vertical stripes that look different from the general streakiness of the plot. Each blue X in the plot corresponds to a particular ion (peak) in a particular MS1 spectrum (vertical slice) that was selected for MS2.



 Display the MS2 spectrum for tetracycline. In the Data Exploration 2D plot, move your cursor around to find the blue X at the retention time and mass for tetracycline that you identified in step 8. When you find the corresponding blue X (at rt ~2.16 min, m/z 443.1428), click on it and wait a few seconds for the Details Panel to update. Scroll down to see the MS2 spectrum that opens.



- Hover your cursor over the top of largest peak in the MS2 spectrum. What is the mass of this fragment?
- To view a plot that shows more detail, click on the View Vector Metabolomics USI button.
 Download an image of the tetracycline MS2 plot by clicking on the Download as PNG or Download as SVG.



- Below (and linked <u>here (https://canvas.pointloma.edu/courses/56791/pages/chemdraw-files-for-week-9-lcms)</u> as a ChemDraw file) is a scheme showing some possible fragmentations of tetracycline in negative ion mode, and the resulting fragment ions. Calculate the exact mass for each of these fragment ions. (*Hint: determine the molecular formula of each fragment; note that they already have a negative charge, so you don't need to determine the conjugate base*. Next, calculate the exact mass corresponding to the formula. If you like, you can return to the virtual desktop and use ChemDraw to open the file and determine the formula and exact mass that way!)
- Using your MS2 data for tetracycline, identify the ion masses in your spectra that could correspond to fragments A, B and C, based on the masses you determined above. Which fragment ion is the most abundant? What does this suggest to you about its stability relative to the other fragments?



- 9. Let's investigate MS2 spectra for two more antibiotics: chloramphenicol and rifamycin.
 - Repeat step 8a-e above to see the MS2 fragmentation spectra for rifamycin and chloramphenicol.
 - The structure of the rifamycin molecular ion is shown below, and in the ChemDraw file <u>here</u> (<u>https://canvas.pointloma.edu/courses/56791/pages/chemdraw-files-for-week-9-lcms</u>), with the most stable fragment shown in blue. In ChemDraw (back on the virtual desktop), use the selection tool to draw a circle around the blue fragment. (Make sure Show Analysis Window is checked under the View menu and that the Exact Mass is set to display at least 4 significant figures before selecting the fragment.) What is the exact mass of this fragment? Does this mass match to a peak in the MS2 spectrum for rifamycin?
 - The structure of chloramphenicol is also shown below, and in the attached ChemDraw file. In ChemDraw, use the selection tool to draw circles around sections of chloramphenicol, checking the Exact Mass of each in the Analysis Window. Propose a fragment that might be responsible for the major peak in the MS2 spectrum of chloramphenicol.



0



chloramphenicol

rifamycin (M-H)⁻ ion probable MS2 fragment shown in blue

Part III: Exploring the LCMS of AH48

For this part, we'll explore data for an extract of AH48 grown at 28 °C in PDB for 5 days - similar to our 'PDB control' extract. Unlike the LCMS data above, this data was collected in **positive ion mode**. As a result, most ions we see will correspond to the molecule plus a proton (M+H)⁺, also known as the conjugate acid.

- 1. Return to the GNPS LCMS Dashboard. (https://gnps-lcms.ucsd.edu/)
- Open the AH48 datafile. Under the File Selection tab, in the GNPS USI field, delete the default file path and paste mzspec:MSV000084951:AH48:scan:1000 into the GNPS USI box under the File Selection tab, then click Link to these plots.
- 3. Let's look at the TIC. Scroll down to the TIC plot. How does it compare to the TIC plot for the antibiotic mixture?
- 4. Save an image of the TIC plot.
- 5. Although the TIC for the crude extract is a bit overwhelming, it is possible to use the XIC to 'go fishing' for molecules that might be in there. To see how this can work with AH48, let's try 'fishing' for a certain molecule that I happen to know is in there we'll call it *Molecule M. Molecule M* has a molecular formula of C₄₉H₇₆N₁₂O₁₄. Determine the exact mass for the conjugate acid of *Molecule M*.
- 6. Paste the exact mass you determined for *Molecule M* into the XIC m/z field to generate an extracted ion chromatogram of only ions of that mass. What do you see? How does this compare to the TIC?
- 7. Let's look at how we can use a **Library Search** and **MASST Search** to compare our MS2 spectrum to MS2 spectra for known molecules, and to unknown molecules whose MS2 spectra are in the GNPS database. (*This is analogous to the KnownClusterBlast and ClusterBlast searches you did in antiSMASH!*)
 - Click on the little red X over the peak to view the MS2 spectrum corresponding to this peak (compound).



 Scroll down to the MS2 spectrum. Click on MASST Spectrum in GNPS. This will open a new tab with the MASST job page in the GNPS website. (Instead of trying to interpret it ourselves, we're going to use a powerful computer to compare this MS2 spectrum against all the other MS2 spectra in the GNPS database!)



 Give your job a name. Mine was "GNPS MASST search of m/z 1057 MS2 spectrum in AH48 ovtract"

💭 Topic Week 1 🗙 🙆	My Drie-Go X 🖬 LOS aduly - X 🗖 GNS-LOS X 🗢 COS https:/ X 🖷 GNS-Maj: X 🗖 GNS-LOS X +
< → C ☆ ● 9	egs wesd edw.ProteoSAFeu'ndex.jp.#Cworkflow' (h20"SEARCH, SINGLE_SPECTRUM'.N20"precursor, mz"/h20"1057.57025366942".h20"Spectrum, string "h20"73.6. 🖈 🛽 🏚
🗄 Apps 📑 Suggested Site	s 🕐 Speenteristent by O 😤 Infectious Causes a 🔯 Vitazionarus Sabuat 🍪 Ann 😭 Disting Resources fau 🚷 Niew Talo 🗖 Week of Providence
	GNPS: Global Natural Products Social Molecular) Networking
	Workflow Selection Search Protocol. None • Reset Firm Save as Protocol. Title: DAPS AM05T search of m/z. 1957 MS2 spectrum in AMM entract.
	Warkflow Description
	SEARCH_SINGLE_SPECTRUM
	Use IMASST to query a single MS-MS spectrum across all public CMPS discosts. The wass spectrometry equivalent of MCBI BLAST helps In put the query spectrum is contact of where else it occurs (including sample information) as well as search a single MSIMS spectrum against all public spectra (Borrien.
	Notifier write resain_35
	Spectrum Input
	Precursor M/2: 1057.57025368
	Spectrum Input: 71.67.428494513120 4233.609375 * 79.69420756978542 40732.34943199275
	(1.)96181362301 874.851307813
R NYT cressword ps_pdf	In the second

- Verify that your email address appears at the bottom of the page, then click Submit. The job will take about 20 minutes to run (during which you can skip ahead to step 8 below; just be sure to come back to finish!)
- When your job is complete, you'll get an email with a link to the completed job page. Open the link to the completed job (or return to the tab if still open).
- At the top, click on View All Library Hits. What known molecule does *Molecule M* match to?
 Scroll all the way to the right to see the structure of this molecule. Right click on the structure to save a copy of the image for your postlab report.

GNPS	Global Natural Products Social Molecular Networking	°-on		
Beck to mein pe	29	_		
Vorkflow	SCAROL_SHIGLE_SPECTRUM (version release_36)			
	D081 [Cone] [Ciene to Labert Versite1] [Viewall Liferary Htts] Community Matches	eleted		
Status	[Dataset Matches] Methods and Clasion for Manuscripts [Viorkflow Written Description]			
	Reanalyze Tilles Found Annian Eline Sound With Ministratic Methodships Production: Sound's Annian			
Hann	[View Foodorics Specific Molecules] enhances distanteenstance/instanteenstance/instanteenstances tableauts			
ALC: NO PERF	emonant (more meneral plant menoral), care men uncered comparty			
Title	GN25 IA35T search of m/z 1057 AS2 spectrum in AH48 entract.			

- 8. Let's go fishing for our Usual Suspects from Week 6 antiSMASH lab.
 - Below is a table of our usual suspects, with the molecular formula given for each. For each, determine the formula and exact mass of the molecular ion peak (M+H)⁺ (aka, the conjugate *acid*) that you would expect to see in *positive* ion mode.
 - Draw the XIC for each molecular ion determined above. Type the exact mass you determined for each usual suspect in the XIC m/z field at the top right of the page, separating each mass with a semicolon. What do you observe in the XIC plot for each suspect? Do any appear as sharp peaks in the XIC (suggesting there is a molecule with that exact mass in the extracts)? Complete the Usual Suspects table below.
 - Seeing a peak with the same exact mass as one of our usual suspects is good support that there is a molecule with that molecular formula, but it doesn't prove that it is the same molecule. What do we call molecules with the same molecular formula, but whose atoms are connected differently?

Usual Suspects

Compound name	Molecular formula	Conjugate acid formula	Conjugate acid exact mass (M+H) ⁺	XIC observations
butirosin	C ₂₁ H ₄₁ N ₅ O ₁₂			
surfactin	C ₅₃ H ₉₃ N ₇ O ₁₃			
bacilysin	C ₁₂ H ₁₈ N ₂ O ₅			
difficidin	C ₃₅ H ₄₈ O ₇			
fengycin	C ₇₂ H ₁₁₀ N ₁₂ O ₂₀			
bacillaene	C ₃₄ H ₄₈ N ₂ O ₆			
macrolactin H	C ₂₂ H ₃₂ O ₅			

Congratulations! You've just done a pretty sophisticated analysis of the raw LCMS data from an extract of AH48 - including looking in the data for known molecules (our so-called Usual Suspects). Next time, we'll learn about a technique that lets us extend our analysis in ways that don't require us to have a hypothesis going into it! See you then!