GBSC 724 Metabolomics Class

Stephen Barnes





Creat	ting .abf fil	les		
Save Location : Browse	Name	Date modified	Туре	Size
Save Converted Files into Same Folder as Raw Files Display Only File Name (Settings)	Neg C4.abf	2/10/2020 4:42 PM	ABF File	609.340 KB
Wilsers)sharmes)Deskton)Class 2020(class 2-10-20 files)Neg. C4 wiff	Neg C4.wiff	3/14/2017 1:54 PM	WIFF File	5.868 KB
:\Users\sbarnes\Desktop\Class 2020\class 2-10-20 files\Neg_C4.wiff	Neg_C4.wiff.scan	3/14/2017 1:54 PM	SCAN File	125,715 KB
:\Users\sbarnes\Desktop\Class 2020\class 2-10-20 files\Neg_C6.wiff	Neg_C5.abf	2/10/2020 4:42 PM	ABF File	574,528 KB
:\Users\sbarnes\Desktop\Class 2020\class 2-10-20 files\Neg_G4.wiff	Neg_C5.wiff	3/14/2017 4:26 AM	WIFF File	6,068 KB
:\Users\sbarnes\Desktop\Class 2020\class 2-10-20 files\Neg_G5.wiff	Neg_C5.wiff.scan	3/14/2017 4:26 AM	SCAN File	118,180 KB
\Users\sbarnes\Desktop\Class 2020\class 2-10-20 files\neg_Go.wiff	Neg_C6.abf	2/10/2020 4:43 PM	ABF File	544,464 KB
	Neg_C6.wiff	3/14/2017 6:02 PM	WIFF File	5,672 KB
	Neg_C6.wiff.scan	3/14/2017 6:02 PM	SCAN File	113,206 KB
	Neg_G4.abf	2/10/2020 4:43 PM	ABF File	624,544 KB
	Neg_G4.wiff	3/14/2017 11:09 AM	WIFF File	6,136 KB
	Neg_G4.wiff.scan	3/14/2017 11:09 AM	SCAN File	129,339 KB
	Neg_G5.abf	2/10/2020 4:44 PM	ABF File	607,292 KB
Clear All Convert Cancel	Neg_G5.wiff	3/14/2017 3:17 PM	WIFF File	6,056 KB
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Completed ×	Neg_G6.abf	2/10/2020 4:44 PM	ABF File	549,515 KB
	Neg_G6.wiff	3/14/2017 3:04 AM	WIFF File	6,092 KB
Raw File Conversion is successfully completed.	Neg_G6.wiff.scan	3/14/2017 3:04 AM	SCAN File	114,121 KB
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Name	Date modified	Туре	Size
MonaRestApi.dll	1/2/2020 12:28 AM	Application extens	35 KB
MonaRestApi.dll.config	4/1/2019 11:03 PM	CONFIG File	2 KB
MSDIAL	1/7/2020 4:12 PM	Application	6,846 KB
MSDIAL.exe.config	9/20/2018 12:40 PM	CONFIG File	2 KB
S MSDIAL	11/24/2019 4:38 PM	Configuration setti	1 KB
MsdialCommon.dll	1/7/2020 4:12 PM	Application extens	45 KB
Ч MsdialConsoleApp	1/7/2020 4:12 PM	Application	132 KB
MsdialConsoleApp.exe.config	7/1/2016 12:39 PM	CONFIG File	1 KB
MsdialDataExporter.dll	5/30/2019 4:29 PM	Application extens	41 KB
Andial Came Dra ages dll	1/7/2020 4:12 DM	Application outons	165 KD



Setting th	e initial parar	nete	ers
Start up a project			
Project file path: C:\Users\sbarnes\Desktop\@	Class 2020\class 2-10-20 files\2020_2_11_7_32_59.mtd	Browse	Select the path to the .abf files
Ionization type			
 Soft ionization (LC/MS, LC/MS/MS, or precu 	ursor-oriented GC/MS/MS)		
Hard ionization (GC/MS)			
Senaration time			
Chromatography (GC, LC, CE, or SFC)			
O ion mobility (now coupled with liquid chron	matography)		
MS method type			
 Conventional LC/MS or data dependent MS 	s/MS		
SWATH-MS or conventional All-ions method	d O All-ions with multiple CEs (cycled like 0V-10V-40V)		
Experiment file:		Browse	
Data type (MS1)	Data type (MS/MS)		
Profile data	Profile data		
O Centroid data	O Centroid data		
lon mode	Target omics		
O Positive ion mode	 Metabolomics 		
Negative ion mode	○ Lipidomics		
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C:\Users\sbarnes\Desktop\Class 2020\class Neg_G6 Sample 1	:\Users\sbarnes\Desktop\Class 2020\cl :\Users\sbarnes\Desktop\Class 2020\cl :\Users\sbarnes\Desktop\Class 2020\cl	ss (Neg_G6	- ·			· · · · · · · · · · · · · · · · · · ·	injeen teraine (pa)	
C\Users\sbarnes\Desktop\Class 2020\class \ Neg_G5 Sample 1 1 2 1 I C\Users\sbarnes\Desktop\Class 2020\class \ Neg_G6 Sample 1 1 3 1 I I C\Users\sbarnes\Desktop\Class 2020\class \ Neg_G6 Sample 1 1 4 1 I <td< th=""><th>C:\Users\sbarnes\Desktop\Class 2020\cla C:\Users\sbarnes\Desktop\Class 2020\class 2020\c</th><th></th><th>Sample</th><th>1</th><th>1</th><th>1</th><th>1</th><th>✓</th></td<>	C:\Users\sbarnes\Desktop\Class 2020\cla C:\Users\sbarnes\Desktop\Class 2020\class 2020\c		Sample	1	1	1	1	✓
C:\Users\sbarnes\Desktop\Class 2020\class Neg_C4 Sample 1 1 3 1 I C:\Users\sbarnes\Desktop\Class 2020\class Neg_C6 Sample 1 1 4 1 I	:\Users\sbarnes\Desktop\Class 2020\cl	ss i Neg_G5	Sample	1	1	2	1	✓
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	Neg_C	4.abf	2/10/	2020 4:42 PM	ABF File	609,34	КВ	
Highlight these files to enter		High	light these	files to er	nter			



Data collection	Peak detection	MS2Dec	Identification	Adduct	Alignment	Mobility	Isotone t	racking	
	reak detection	10152000	lucification	Adddet	Alignment	WODINTy	Botope	acking	
Mass accuracy									
MS1 toleran	ce:					0.0	1 Da		
MS2 toleran	ce:					0.01	5 Da		
							X		
Advanced							Ch	anged	

No changes to these tabs	
–	
Analysis parameter setting —	
Data collection Peak detection MS2Dec Identification Adduct Alignment Mobility Isotope tracking	
Peak detection parameters	
Minimum peak height: 1000 amplitude	
Mass slice width: 0.1 Da	
(v) Advanced	
C. La Contraction of the Contrac	
Analysis parameter setting	
Data collection Peak detection MS2Dec Identification Adduct Alignment Mobility Isotope tracking	
Deconvolution parameters	
Sigma window value: 0.5	
MS/MS abundance cut off: 0 amplitude	
✓ Advanced	

Analysis par									
Data collection	Peak detection	MS2Dec	Identification	Adduct	Alignment	Mobility	Isotope tr	acking	
MSP file and M	1S/MS identificati	on setting							
MSP file:								Select	
Retention tir	me tolerance:						100 mir	1	
Accurate ma	ss tolerance (MS	1):							
Accurate ma	ss tolerance (MS	2):					0.05 Da		
Identification	n score cut off:						80 %		
Use retentio	n time for scoring	g:							
Use retentio	n time for filterin	g:							

Ҷ Analysis par	ameter setting								×	
Data collection	Peak detection	MS2Dec	Identification	Adduct	Alignment	Mobility	Isotop	oe tracki	ng	
MSP file and N	MS/MS identification	n setting								
MSP file:	C:\Users\sbarnes\	Desktop\	Databases\MSN	AS-Public	-Neg-VS14.m	nsp			Select	
Retention ti	me tolerance:						100	min		
Accurate ma	ass tolerance (MS1)):					0.01	Da		
Accurate ma	ass tolerance (MS2)):				(0.015	Da		
Identificatio	n score cut off:						80	%		
Use retentio	on time for scoring:								This I	has been rese
Use retentio	on time for filtering	:								

Analysis paramete	r setting						—		\times	
ata collection Peak	detection	MS2Dec	Identification	Adduct	Alignment	Mobility	Isotope	tracking		
Adduct ion setting							User-	User-defined adduct		
Molecula	r species		Charge		Accurate	e mass [Da]		Inclu	ded	
[M-H]-		1		-1.00	782503207				/	
[M-H2O-H]-		1	[-19.0	1838971207				/	
[M+Na-2H]-		1	1	20.97	411921676				/	
[M+CI]-		1		34.96	885268					
[M+K-2H]-		1		36.94	805661586					
[M+FA-H]-		1		44.99	765396793				/	
[M+Hac-H]-		1		59.01	330396793					
[M+C2H3N+Na-2H	-	1		62.00	066831777					
[M+Br]-		1		78.91	83371					
[M+TFA-H]-		1		112.9	8503896793					
[M-C6H10O4-H]-		1		-147.	06573383101					
[M-C6H10O5-H]-		1		-163.	06064845057					
[M-C6H8O6-H]-		1		-177.	03991300599	6				
[M+CH3COONa-H]		1		80.99	524996793					
[2M-H]-		1		-1.00	782503207				/	
[2M+FA-H]-		1		44.99	765396793				/	
[2M+Hac-H]-		1		59.01	330396793					
[3M-H]-		1		-1.00	782503207				/	
[M-2H]2-		2	2	-2.01	565006414				/	
[M-3H]3-		3	3	-3.02	347509621				/	













🐫 Aligni	ment Table										>
Num of r	ows: 81	Meta	abolite Name	Filter	C	omment Filter		61.8	1 Mz Range	997.29	0.1 RT Range 26.
	C C	Set									U U
ID	RT(min)	m/z	Туре	Fill %	Metabolite name	Comment	Correlation	S/N	ANOVA P-value	Fold change (Max/Min)	BarChart
1702	24.43	325.1866	[M-H]-	0.33	Dodecylbenzenesulfonic		-0.57	754.0	4.66E-01	1.05	
2187	23.10	362.9694	[M-H]-	0.83	Perfluoroheptanoic acid;		0.16	154.6	8.67E-01	1.08	
2333	13.04	375.1297	[M-H]-	1.00	RIBOFLAVIN		0.61	12223.1	4.44E-01	1.13	
2335	12.47	375.1349	[M-H]-	0.17	(-)-Riboflavin; LC-ESI-QT		-0.57	287.7	3.86E-01	1.38	
2829	24.62	412.9666	[M-H]-	1.00	Perfluorooctanoic acid; l		0.25	582.5	8.85E-01	1.07	
3020	23.75	426.9655	[M-H]-	0.83	6:2 Fluorotelomer sulfor		0.48	1551.0	4.68E-01	1.43	
3079	18.07	431.2114	[M-H]-	1.00	5-hydroxy-2,2,6,6-tetran		-0.33	1927.8	5.77E-01	1.29	
3087	16.59	432.2042	[M+FA-H]-	1.00	Guan-fu base Y		-0.54	534.6	1.69E-01	1.67	
3235	16.34	445.0759	[M-H]-	0.50	apigenin-7-O-glucuroni		0.80	4377.8	2.65E-01	166.64	
	15.56	445.0761	[M-H]-	0.83	apigenin-7-O-glucuroni		1.00	28256.1	8.87E-03	164.09	
3236	15.50										

All the ions in the table can be highlighted, copied and transferred to Excel

	C	D	E	F	G	H	1	J	K
ID TRT	Mass M	Ion 💌	Fractior *	×		Correl	s/N *	p-value *	FC 💌
20 26.0	3 96.96125	[M-H]-	1	Phosphoric acid		-0.4677	5 70.35927	0.870505	1.046085
21 17.9	5 96.96135	[M-H]-	0.333333	Phosphoric acid		-0.2258	1 11.09236	0.5714	1.266831
44 14.4	2 121.0303	[M-H]-	1	2-Hydroxybenzaldehyde		-0.0208	4 230.3153	0.392505	1.58231
56 7.7	6 129.0212	[M-H]-	1	CITRACONIC ACID		0.20701	5 89.03239	0.344172	1.530682
57 8.2	6 129.0212	[M-H]-	0.333333	CITRACONIC ACID		0.43511	3 34.15426	0.218116	1.594494
63 10.0	7 131.0361	[M-H]-	0.333333	Glutaric acid; LC-ESI-QTOF; MS2; CE		0.61115	4 81.5596	0.644436	1.588785
84 12.1	2 144.0458	[M-H]-	1	4-Hydroxyquinoline		0.96806	2 268.3839	0.609065	1.246376
85 17.1	4 144.0478	[M-H]-	1	4-Hydroxyquinoline		0.47071	9 13043.28	0.380413	1.298935
86 18.6	4 144.048	[M-H]-	0.5	4-Hydroxyquinoline		0.28564	2 118.1601	0.333711	1.610268
95 17.9	2 148.0242	[M-H]-	1	Benzyl Isothiocyanate		0.08763	7 150.4269	0.67476	1.374674
96 13.2	1 149.0088	[M-H]-	0.166667	L-(+)-tartaric acid; LC-ESI-QTOF; MS2; CE		-0.4297	8 19.05287	0.4837	1.8518
97 25.6	6 149.0106	[M-H]-	0.333333	(R.R)-TARTARIC ACID		-0.4320	3 52.50727	0.54429	1.590465
		սերն	ie colui	mns to make it easier to apprecia	ate th	e data			
		n up ti	ie colui	nns to make it easier to apprecia	te th	e data			
ID • RT	• Mass		Fractic •	nns to make it easier to apprecia	ite th	e data	* S/N *	p-valu *	FC 🔻
ID v RT 20 26.	 Mass 96.961 	I ION 3 [M-H]-	Fractic + 1.00	nns to make it easier to apprecia	ite th	 Corre -0.467 	• S/N •	p-valu ▼ 5 0.87050	FC -
ID • RT 20 26. 21 17.	Mass 96.961 95 96.961	Ion 3 [M-H]- 4 [M-H]-	 Fractic * 1.00 0.33 	Phosphoric acid	ite th	e data Corre -0.467 -0.225	 S/N 3 70.36 3 11.09 	p-valu ▼ 5 0.87050 9 0.57140	FC - 1.05 1.27
ID R T 20 26. 21 17. 44 14.	 Mass 96.961 96.961 121.030 	Ion 3 [M-H]- 4 [M-H]- 3 [M-H]-	 Fractic + 1.00 0.33 1.00 	Phosphoric acid 2-Hydroxybenzaldehyde	ate th	 Corre -0.467 -0.225 -0.020 	 S/N 70.36 11.09 230.32 	p-valu ▼ 0.87050 0.57140 2 0.39251	FC 1.05 1.27 1.58
ID • RT 20 26. 21 17. 44 14. 56 7.	 Mass 96.961 96.961 121.030 129.021 	Ion 3 [M-H]- 4 [M-H]- 3 [M-H]- 2 [M-H]-	Fractic - 1.00 0.33 1.00 1.00	Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID	ate th	 Corre -0.467 -0.225 -0.020 0.2070 	 S/N TO.36 11.09 230.32 89.03 	p-valu ▼ 5 0.87050 0.57140 2 0.39251 3 0.34417	FC 1.05 1.27 1.58 1.53
ID • RT 20 26. 21 17. 44 14. 56 7. 57 8.	Mass 23 96.961 95 96.961 42 121.030 76 129.021 26 129.021	 Ion 3 [M-H]- 4 [M-H]- 3 [M-H]- 2 [M-H]- 2 [M-H]- 	 Fractic 1.00 0.33 1.00 1.00 0.33 	Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID CITRACONIC ACID	ate th	 Corre -0.467 -0.225 -0.020 0.2070 0.435: 	 S/N 70.38 11.09 230.33 89.03 34.15 	 p-valu o.87050 o.57140 o.39251 o.34417 o.21812 	FC 1.05 1.27 1.58 1.53 1.59
ID • RT 20 26. 21 17. 44 14. 56 7. 57 8. 63 10.	 Mass 96.961 96.961 121.030 129.021 129.021 129.021 131.036 	Ion I Ion Ion Ion Ion Ion Ion Ion Ion	 Fractic 1.00 0.33 1.00 1.00 0.33 0.33 0.33 	Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID CITRACONIC ACID Gilutaric acid; LC-ESI-QTOF; MS2; CE	ite th	 Corre -0.467 -0.225 -0.020 0.2070 0.435: 0.6112 	 S/N TO.36 11.09 230.32 89.09 34.19 81.56 	 p-valu ▼ 0.87050 0.57140 0.39251 0.34417 0.21812 0.64444 	FC 1.05 1.27 1.58 1.53 1.59 1.59
ID • RT 20 26. 26. 21 17. 44 56 7. 57 63 10. 84	 Mass 96.961 96.961 121.030 129.021 129.021 131.036 144.045 	Ion I Ion I [M-H]- [M-H]- [M-H]- [M-H]- [M-H]- [M-H]- [M-H]- [M-H]- [M-H]- [M-H]-	 Fractic 1.00 0.33 1.00 0.33 0.33 0.33 1.00 	Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID Glutaric acid; LC-ESI-QTOF; MS2; CE 4-Hydroxyquinoline	ite th	 Corre -0.467 -0.225 -0.020 0.2070 0.435: 0.6112 0.968: 	 S/N 70.33 11.09 230.33 89.03 34.15 81.56 268.38 	 p-valu ▼ 0.87050 0.57140 0.39251 0.34417 0.21812 0.64444 0.60907 	FC 1.05 1.27 1.58 1.53 1.59 1.59 1.25
ID • RT 20 26. 21 17. 44 14. 56 7. 57 8. 63 10. 84 12. 85 17.	 Mass 96.961 96.961 121.030 129.021 129.021 131.036 144.045 144.047 	I Ion 3 [M-H]- 4 [M-H]- 3 [M-H]- 2 [M-H]- 2 [M-H]- 2 [M-H]- 8 [M-H]- 8 [M-H]-	 Fractic ~ 1.00 0.33 1.00 0.33 0.33 0.33 1.00 1.00 1.00 	Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID Glutaric acid; LC-ESI-QTOF; M52; CE 4-Hydroxyquinoline 4-Hydroxyquinoline	ite th	 Corre -0.467 -0.225 -0.020 0.2070 0.4353 0.6111 0.9683 0.4703 	 S/N 70.33 11.09 230.33 89.00 34.19 81.56 268.38 13043.28 	p-valu ▼ 5 0.87050 9 0.57140 2 0.39251 3 0.34417 5 0.64444 8 0.60907 8 0.38041	FC 1.05 1.27 1.58 1.53 1.59 1.59 1.25 1.30
ID * RT 20 26. 21 21 17. 44 14. 56 7. 57 8. 63 10. 84 12. 85 17. 9. 86 18.	 Mass 96.961 96.961 121.030 129.021 129.021 131.036 144.045 144.047 144.048 	 Ion IM-H]-4 IM-H]-4 IM-H]-2 IM-H]-2 IM-H]-1 IM-H]-8 IM-H]-9 IM-H]-90 IM-H]-1 	 Fractic + 1.00 0.33 1.00 0.33 0.33 1.00 1.00 1.00 0.50 	Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID CITRACONIC ACID Glutaric acid; LC-ESI-QTOF; MS2; CE 4-Hydroxyquinoline 4-Hydroxyquinoline	ite th	 Correction -0.467 -0.225 -0.020 0.2070 0.4353 0.6112 0.9683 0.4703 0.2850 	 S/N 70.36 11.06 230.33 89.03 34.15 81.56 268.33 13043.28 118.16 	 p-valu v 0.87050 0.57140 0.39251 0.34417 0.21812 0.64444 0.60907 0.38041 0.33371 	FC 1.05 1.27 1.58 1.53 1.59 1.59 1.25 1.30 1.61
ID • RT 20 20 26. 21 17. 44 14. 56 7. 57 8. 63 10. 84 12. 85 17. 86 18. 95 17.	 Mass 96.961 96.961 121.030 129.021 129.021 131.036 144.045 144.047 144.048 148.024 	2 Ion 3 [M-H]- 4 [M-H]- 3 [M-H]- 2 [M-H]- 2 [M-H]- 1 [M-H]- 8 [M-H]- 8 [M-H]- 9 [M-H]- 9 [M-H]- 2 [M-H]-	 Fractic 1.00 0.33 1.00 0.33 0.33 0.33 1.00 1.00 0.50 1.00 	Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID Gilutaric acid (LC-ESI-QTOF; MS2; CE 4-Hydroxyquinoline 4-Hydroxyquinoline 4-Hydroxyquinoline	ete th	 Corre -0.467 -0.225 -0.020 0.2070 0.4635 0.6112 0.9685 0.4700 0.2856 0.08700 	 S/N 70.36 11.05 230.33 89.03 34.15 268.33 13043.24 118.16 150.43 	 p-valu v 0.87050 0.57140 0.39251 0.34417 0.21812 0.64444 0.60907 0.38041 0.33371 0.67476 	FC 1.05 1.27 1.58 1.53 1.59 1.59 1.59 1.25 1.30 1.61 1.37
ID * RT 20 26. 21 17. 44 16. 57 8. 63 10. 84 12. 85 17. 86 18. 95 17. 96 13.	 Mass 96.961 96.961 121.030 129.021 129.021 131.036 144.045 144.044 144.044 148.024 149.008 	 Ion 3 (M-H)- 4 (M-H)- 3 (M-H)- 2 (M-H)- 2 (M-H)- 1 (M-H)- 8 (M-H)- 8 (M-H)- 2 (M-H)- 8 (M-H)- 	 Fractic * 1.00 0.33 1.00 0.33 0.33 1.00 0.33 1.00 0.50 1.00 0.17 	Phosphoric acid Phosphoric acid Phosphoric acid 2-Hydroxybenzaldehyde CITRACONIC ACID Glutaric acid; LC-ESI-QTOF; MS2; CE 4-Hydroxyquinoline 4-Hydroxyquinoline Benzyl Isothiocyanate L-(4)-tartaric acid; LC-ESI-QTOF; MS2; CE	ete th	 Corre -0.467 -0.225 -0.020 0.0207 0.435: 0.611: 0.968: 0.470: 0.2856 0.0857 0.0857 0.0856 	 S/N S/N 3 T1.09 230.32 230.32 240.11 268.33 13043.224 118.16 150.43 19.02 	 p-valu × 0.87050 0.57140 0.32451 0.32451 0.34417 0.21812 0.64444 0.60907 0.33071 0.33371 0.67476 0.48370 	FC - 1.05 1.27 1.53 1.59 1.59 1.25 1.30 1.61 1.37 1.85

Reorganizing the data according to RT

	RT	Mass	Ion	Fraction		Correl	S/N	p-value	FC
258	6.51	191.0212	[M-H]-	0.67	CITRATE	0.0265	59.66	0.51275	1.29
677	6.51	243.0623	[M-H]-	0.83	Pseudouridine	0.6598	57.52	0.34761	1.37
678	7.37	243.0630	[M-H]-	0.50	Pseudouridine	0.4058	48.79	0.38523	1.53
259	7.37	191.0213	[M-H]-	0.17	Citric acid	-0.0381	25.39	0.63893	1.23
56	7.76	129.0212	[M-H]-	1.00	CITRACONIC ACID	0.2070	89.03	0.34417	1.53
167	7.76	173.0114	[M-H]-	1.00	cis-Aconitate	0.2025	231.84	0.37510	1.51
57	8.26	129.0212	[M-H]-	0.33	CITRACONIC ACID	0.4351	34.15	0.21812	1.59
1151	8.81	283.0680	[M-H]-	0.50	Xanthosine; LC-ESI-QTOF; MS2; CE	0.4278	74.18	0.49296	1.53
1356	8.93	296.1021	[M-H]-	1.00	N2-Methylguanosine	0.5310	330.68	0.49288	1.36
1565	9.83	310.1140	[M-H]-	1.00	N2,N2-Dimethylguanosine	0.2243	264.50	0.51888	1.36
460	9.96	218.1030	[M-H]-	0.83	D-PANTOTHENIC ACID	-0.5419	603.07	0.49295	1.91
63	10.07	131.0361	[M-H]-	0.33	Glutaric acid; LC-ESI-QTOF; MS2; CE	0.6112	81.56	0.64444	1.59
114	11.44	157.0404	[M-H]-	0.33	ALLANTOIN	0.5857	23.50	0.41526	1.4

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Organizing the data according to p-value

RT	Mass	Ion	Fraction		Correl	S/N	p-value	FC	
15.57	269.0468	[M-H]-	0.50	Emodin	-0.5117	2344.65	0.00537	81.25	
15.56	445.0761	[M-H]-	0.83	apigenin-7-O-glucuronide	-0.5247	28256.12	0.00887	164.09	*
16.89	167.1055	[M-H]-	0.83	Chrysanthemic Acid	0.3701	115.90	0.01392	1.35	
16.07	204.0672	[M-H]-	1.00	Indolelactic acid	0.1934	5353.15	0.02399	1.41	
16.07	158.0597	[M-H]-	1.00	Indole-3-acetaldehyde; LC-ESI-QTOF; MS2; CE	0.1690	110.97	0.09263	1.49	
17.62	174.0565	[M-H]-	1.00	Indoleacetic acid; LC-ESI-QTOF; MS2; CE	0.4052	2283.56	0.11465	1.92	
21.80	311.1691	[M-H]-	1.00	Triptophenolide	-0.7362	2340.87	0.14646	1.16	
14.14	193.0354	[M-H]-	0.67	Glucuronate	0.8812	134.13	0.16489	1.42	
22.60	311.1691	[M-H]-	1.00	Triptophenolide	-0.9200	1109.05	0.16520	1.14	
15.21	165.0575	[M-H]-	0.33	3-(3-Hydroxyphenyl)propionic acid	0.0486	235.63	0.17098	12.25	•
19.69	325.1794	[M-H]-	0.17	Dodecylbenzenesulfonic acid	-0.5846	427.25	0.17189	1.26	
8.26	129.0212	[M-H]-	0.33	CITRACONIC ACID	0.4351	34.15	0.21812	1.59	These are probably
18.02	269.0439	[M-H]-	1.00	Apigenin; LC-ESI-QTOF; MS2; CE	-0.4217	7616.71	0.22022	11.24	
12.56	621.1078	[M-H]-	0.50	4'-O-GlcA-7-O-GlcA Apigenin (NMR)	-0.4362	3665.59	0.23690	130.34	🖌 🖉 genistein metabolit
16.89	204.0664	[M-H]-	1.00	N-Cinnamoylglycine	0.4430	2884.94	0.26166	2.43	_ //
21.40	193.0399	[M-H]-	1.00	D-(+)-Galacturonic acid	0.7855	412.51	0.26947	1.50	
18.02	432.2045	[M+FA-H]-	0.33	Guan-fu base Y	0.7312	615.06	0.26950	1.52	_ //
16.95	187.0982	[M-H]-	1.00	Azelaic acid (Not validated); PlaSMA ID-221	0.5178	3418.23	0.27544	1.31	1
20.53	269.0465	[M-H]-	0.83	Aloe-emodin	-0.4072	2886.64	0.28086	38.19	7
19.72	459.0892	[M-H]-	0.50	oroxindin	-0.4125	955.03	0.28982	43.35	
18.64	144.048	[M-H]-	0.50	4-Hydroxyquinoline	0.2856	118.16	0.33371	1.61	
25.48	157.039	[M-H]-	0.50	ALLANTOIN	0.5537	20.31	0.34204	1.63	
7.76	129.0212	[M-H]-	1.00	CITRACONIC ACID	0.2070	89.03	0.34417	1.53	
25.69	325.1855	[M-H]-	1.00	Dodecylbenzenesulfonic acid	0.9645	3118.95	0.34721	1.31	
6.51	243.0623	[M-H]-	0.83	Pseudouridine	0.6598	57.52	0.34761	1.37	
11.45	181.0501	[M-H]-	0.83	DL-3-(4-Hydroxyphenyl)lactic acid; LC-ESI-QTOF; MS2; CE	-0.1964	85.72	0.35649	1.50	
24.04	265.1473	[M-H]-	1.00	C12-AS (TENTATIVE)	-0.4008	1845.40	0.35770	2.09	
14.54	191.0211	[M-H]-	0.33	CITRATE	0.5951	40.59	0.37175	1.74	





Set up	for PCA analysis
🐫 PCA setting	- 🗆 ×
Maximum principal component:	5
Scale method:	Pareto scale v
Transform method:	None v
Metabolite selection	
MS2 matched MS1 matach	ed 🗌 Unknown
ote – selecting the known compounds	Done





Cataon fan	
Set up for	PLSDA analysis
Setting	- 🗆 ×
General setting	Components number
Scale method:	Pareto scale ~
Transform method:	None ~
PLS method	
● PLSDA ○ PLSR	O OPLSDA O OPLSR
Metabolite selection	
✓ MS2 matched □ MS	IS1 matached 🗌 Unknown
 (1) Set Y (response) variables (2) For (O)PLS-DA, use a bina * Non-zero values are recogi (3) For (O)PLS-R, set sequent 	es at menu->option->file property. hary (0 or 1) value as the response. gnized as 1 in (O)PLS-DA testing. ntial values.
	Done





PLS setting	- 0	\times	
General setting	Components nun	nber	
Auto fit		2	
Scale method:	Pareto scale	~	
Transform method:	None	~	
PLS method O PLSDA O PLSR Metabolite colortion	● OPLSDA ○ OPLSF	R	analysis
metabolile selection			
MS2 matched MS1	matached 🗌 Unknow	wn	
MS2 matched MS2 matched MS1 (1) Set Y (response) variables i (2) For (O)PLS-DA, use a binar * Non-zero values are recogni (3) For (O)PLS-R, set sequentia	at menu->option->file y (0 or 1) value as the r ized as 1 in (O)PLS-DA al values.	wn property. response. testing.	













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Export option		
File: alignmentResult_2020_2_11	_7_39_25	
Raw data matrix (Height)	Peak ID matrix	
 Normalized data matrix 	Retention time matrix	
Raw data matrix (Area)	m/z matrix	
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Filtered by blank neaks (must be sh	neked in alignment parameter setting)	metabolite neaks, in each sample
Filtered by blank peaks (must be ch	eckea in augnment parameter setting)	metabolite peaks in each sample
	s of blank samples	
Missing value option		
Replace zero values with 1/10	of minimum peak height over all samples	
Isotope labeled tracking option	Target file	
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	Evnort Cancel	
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File: alignmentResult_2020_2_11_	.7_39_25 ×	
Raw data matrix (Height)	Peak ID matrix	
Normalized data matrix	Retention time matrix	
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*Export as mztab-M	MS/MS included matrix	This output file allows us to look at
	GNPS export	This output me anows us to look at
	S/N matrix export	adduct ions that are related to the
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Filtered by blank peaks (must be che	ecked in alignment parameter setting)	
Filtering by the ion abundances	s of blank samples	
Missing value option		
Replace zero values with 1/10 c	of minimum peak height over all samples	
sotope labeled tracking option	Target file	
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