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▶ To cite this version:

Axel Raux, Lauriane Rambaud, Anne-Lise Royer, Justine Massias, Yann Guitton, et al.. GC-HRMS processing tools for untargeted metabolomics. Metabolomics 2022, Jun 2022, Valencia, Spain. 2022. hal-04264200

HAL Id: hal-04264200 https://hal.science/hal-04264200

Submitted on 30 Oct 2023

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GC-HRMS PROCESSING TOOL FOR UNTARGETED METABOLOMICS

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ALIMENTATION AU COEUR DE LA VIE







Introduction

Metabolomics and related sciences use a combination of analytical and statistical approaches to

NEED OF ANOTHER SOLUTION



Automatic spectral matching with TraceFinder[™] software

qualitatively and quantitatively analyze small molecules present in a biological system to answer biological questions. Metabolomics and untargeted studies require the maximization of compounds detected in the analysis, while the results must be reproducible and robust. Gas chromatographymass spectrometry (GC-MS) is a commonly used technology in metabolomics research thanks to the high sensitivity and high throughput, as well as the wealth of databases. The use of an internal database in essential in metabolomics to improve and fasten biomarker annotation, as well as giving biological meaning to biomarkers. Database creation can be done with mainly two ways : either manual, or automatic, with the use of software, like TraceFinder that we have evaluated.

Experimental



Thermo Scientific software for LC-MS or GC-MS data processing



5	Status	Sample name	Sample Id	^	L
	li.	20200123_016	047G-I- 10 ng/uL-MOX-MSTFA		l
	lala.	20200123_017	049I-I- 10 ng/uL-MOX-MSTFA		l
	L.	20200123_018	149I-I- 10 ng/uL-MOX-MSTFA		l
	lala.	20200123_019	093I-I- 10 ng/uL-MOX-MSTFA		l
	l.l.	20200123_020	092I-I- 10 ng/uL-MOX-MSTFA		l
	lala.	20200123_021	199I-I- 10 ng/uL-MOX-MSTFA		b
	l.l.	20200123_022	049G-I- 10 ng/uL-MOX-MSTFA		ľ
	lala.	20200123_023	147I-I- 10 ng/uL-MOX-MSTFA		
	li.	20200123_024	Mix metabo 2 - 10 ng/uL-MOX-MSTFA		
				~	
	cition d	ate: 1/2//2020 /-/1	00 0.0		1

	^		Component Name	RT	Ref m/z	Area	Height 🔍	TIC
MOX-MSTFA		•	Citric acid, 4TMS derivati	13.372	273.096985	12531881719	4294752334	15951129706
IOX-MSTFA			Sebacic acid, di(2-octyl)	25.296	185.117203	6776181930	2494702144	8083925702
IOX-MSTFA			Terephthalic acid, 2TMS	12.983	295.080780	2779771479	850802225	2477280282
IOX-MSTFA			Palmitoleic acid TMS	16.630	117.036568	1183393721	434830083	1742627107
IOX-MSTFA			Peak@15.48298	15.483	147.065521	1038999645	397000846	3232015854
IOX-MSTFA			2,3'-Dipyridyl	9.177	156.067886	3553127983	396659407	1010654985
MOX-MSTFA	Ľ	-	1-(4-Pyridyl)-4-pyridone	17.178	144.068207	1331827673	311340133	1155766233
IOX-MSTFA			Peak@8.51989	8.520	180.062866	729293246	281732511	1170847583
ng/uL-MOX-MSTFA			(Z)-Docos-9-enenitrile	22.104	122.096512	706781174	254613156	3565467478
	~		Sucrose, 8TMS derivative	23.531	361.168488	469783334	172766452	683833364
>	-	7	Peak@12.39582	12.396	140.031677	481083036	161521431	525115407
	~		Peak@16.01283	16.013	293.192505	403261868	151374939	587874427
			Squalene	25.467	81.069969	400960157	145747338	749064922
od\Omique-002.metł			Phthalic acid, hex-3-yl is	14.077	149.023392	326713451	114102058	149482647
	~							

Peak identification and NIST library comparison



F	C 1 1	A 1	6 I.I.							
	Status	Sample name	Sample Id							
20200123_010		20200123_016	047G-I- 10 ng/uL-MOX-MSTFA							
	I II.	20200123_017	049I-I- 10 ng/uL-MOX-MSTFA							
		20200123_018	149I-I- 10 ng/uL-MOX-MSTFA							
	Line	20200123_019	093I-I- 10 ng/uL-MOX-MSTFA							
		20200123_020	092I-I- 10 ng/uL-MOX-MSTFA							
	Labor	20200123_021	199I-I- 10 ng/uL-MOX-MSTFA							
		20200123_022	049G-I- 10 ng/uL-MOX-MSTFA							
•	L	20200123_023	147I-I- 10 ng/uL-MOX-MSTFA							
		20200123_024	Mix metabo 2 - 10 ng/uL-MOX-MSTF/							
<										
A	uisition d	ate: 1/24/2020 4.41.00								
ncq	Acquisition date: 1/24/2020 4:41:00 AM									
nst	InstrumentName type:Q Exactive GC Orbitrap									
nst	ISE ID: EXACTIVE Series SIOE 143									
Sam	nle type	Unknown	CA-15-INT IO(Method/Offilque-002.III							
ample type: Unknown										

WHAT ARE THE NEXT STEPS ?



Manual spectral matching with external GC-EI-MS databases

EIC collection...



<u>.</u>	· 🚽 •						
	Active	Measured m/z	Area	Height	Mass error (ppm)	Fragment count	Mass Export Type
Þ	\checkmark	273.096985	12531881719	4294752334		0	TargetCompound
		147.065521	5241768578	1758814778		0	ConfirmingAndFragment
	\checkmark	183.047058	3003440104	1048892126		0	ConfirmingAndFragment
		347.115845	2297099449	781831870		0	ConfirmingAndFragment
	\checkmark	257.065887	2108290711	733956549		0	ConfirmingAndFragment
		149.044769	2129951721	709334133		0	ConfirmingAndFragment
	L 21	211 021 /02	10108959991	551182051			Nene

Compound database obtained with TraceFinder Internal database GC-EI-HRMS of MOX/MSTFAderivatized metabolites Erucic acid, TMS Fructose-6-phosphate, MEOX-6TMS

Glucose 5TMS 1MOX Glutamic acid, 3TMS Glutamine, 3TMS Glycerol 2-Phosphate 4TMS

Inosine, 4TMS derivative Itaconic acid, 2TMS L-Kynurenine, 3TMS L-Phenylalanine, 2TMS

Indole-2,4,5,6,7-d5-3-acetic acid, 2TMS Indole-2.4.5.6.7-d5-3-acetic acid. TM

Heptadecane Hexatriacontane] Hippuric acid, TMS Indol-3-acetic acid, 2TMS

ane											
pound e	Peak Label	Peak Workflow	ņ	Associated Target Peak	ņ	Chemical Formula	MS Order		Precursor m/z	Product m/z	⊨ m/z +=
hydroxy-D-pro	T1F2: 147.06554	Fragment	•	T1: 230.13886	•		ms1	/ -	0.000	0.000	147.06554
hydroxy-D-pro	T1F3: 231.14185	Fragment	•	T1: 230.13886	•		ms1	-	0.000	0.000	231.14185
hydroxy-D-pro	T1F4: 140.08896	Fragment	•	T1: 230.13886	•		ms1	•	0.000	0.000	140.08896
hydroxy-D-pro	T1F5: 149.04479	Fragment	•	T1: 230.13886	•		ms1	•	0.000	0.000	149.04479
hydroxy-D-pro	T1F6: 304.15762	Fragment	•	T1: 230.13886	•		ms1	•	0.000	0.000	304.15762
acid, 4TMS	T1: 273.09699	TargetPeak	•		•		ms1	•	0.000	0.000	273.09699
acid, 4TMS	T1C1: 257.06589	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	257.06589
acid, 4TMS	T1C2: 347.11585	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	347.11585
acid, 4TMS	T1C3: 183.04706	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	183.04706
ic acid, 4TMS	T1C4: 147.06552	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	147.06552
Citric acid, 4TMS	T1C5: 149.04477	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	149.04477
Citric acid, 4TMS	T1C6: 211.04179	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	211.04179
Citric acid, 4TMS	T1C7: 375.11069	Confirming	•	T1: 273.09699	•		ms1	•	0.000	0.000	375.11069
Citric acid, 4TMS	T1F1: 347.11585	Fragment	•	T1: 273.09699	-		ms1	•	0.000	0.000	347.11585
Citric acid, 4TMS	T1F2: 183.04706	Fragment	•	T1: 273.09699	-		ms1	•	0.000	0.000	183.04706
Citric acid, 4TMS	T1F3: 147.06552	Fragment	•	T1: 273.09699	•		ms1	•	0.000	0.000	147.06552
Citric acid, 4TMS	T1F4: 257.06589	Fragment	•	T1: 273.09699	•		ms1	•	0.000	0.000	257.06589
Citric acid, 4TMS	T1F5: 149.04477	Fragment	•	T1: 273.09699	•		ms1	•	0.000	0.000	149.04477
Citric acid, 4TMS	T1F6: 211.04179	Fragment	•	T1: 273.09699	•		ms1	•	0.000	0.000	211.04179
Citric acid, 4TMS	T1F7: 375.11069	Fragment	•	T1: 273.09699	•		ms1	•	0.000	0.000	375.11069
Cystathionine 4TMS	T1: 218.10234	TargetPeak	•		•	C19H46N2O4SSi4	ms1	•	0.000	0.000	218.10234
Cystathionine 4TMS	T1C1: 160.06094	Confirming	•	T1: 218.10234	•	C19H46N2O4SSi4	ms1	•	0.000	0.000	160.06094
Vistathionine ATMS	T1C2: 147 06551	Confirming	-	T1-218 10234	-	C10H46N2O45Si4	mc1	-	0.000	0.000	147.06551

PROS	CONS
 Fast Reproducible Automatic All-in-one solution 	 Nothing better than human experience ^(C) Consequent data volume needed Not free of charge

Library spectrum



• Human eye assessment

Fastidious

• Time consuming

• Reproducibility

Conclusion

Internal databases are of great interest for annotation pipeline in metabolomics and lipidomics. The addition of GC-EI-HRMS spectra appended a further annotation level for metabolites identification. In order to create the database, the « manual » solution seems to be the most available and easy to use, but also the most fastidious one. On the other hand, many softwares now offer help in database creation from raw data. TraceFinder software, provided by Thermo Scientific, was evaluated here and helped us to fastly and easily build our internal metabolites database. The GC-EI-HRMS database, now composed of 51 standards, which keeps expanding, will be used later for features annotation in different metabolomics projects involving several matrices (urine, blood, milk, tissue, etc...). Other softwares, preferably free of charge, will be assessed for database creation.

database