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UNTARGETED METABOLOMIC APPROACH BY GC-QTOF : FROM LOW TO HIGH RESOLUTION

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Untargeted metabolomic approach aims to gather information on as many metabolites as possible in biological systems by taking into account all informations present in the data sets. This approach is essential to pinpoint modifications of metabolic pathways associated to nutritional, health or environmental status for identifying biomarkers.

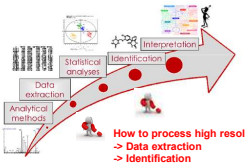
GC is often coupled to single quadrupole analyzers, which offer high sensitivity, good dynamic range but operate with slower scan rates and lower resolution compared to Time of Flight (ToF) systems. GC coupled with ToF Mass Spectrometry is increasingly used for metabolic profiling because of fast acquisition rates, particularly useful for an accurate deconvolution of overlapping peaks obtained from complex mixtures.

GC-MS 5975 Agilent

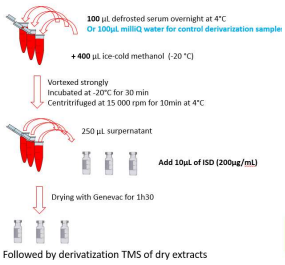
	Low Resolution	High Resolution
Sensivity	-	+
Resolution	-	+
Acquisition Rate	+	++
Databases	NIST, GOLM, FIEHN and in-house databases	MassBank and in-house databases

GC-QTof 7200 Agilent

Untargeted metabolomic approach

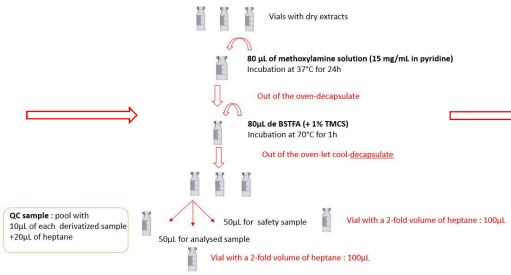


Sample preparation*



*Adapted from Gao X. *et al*, *Anal Chem* 2010

Trimethylsilylation Derivatization*



GCMS Analysis*

GC Conditions :

Injection system: 2 µL split 1/20, T = 250 °C.

Column: HPS-MS (30 m x 0.25 mm x 0.25 µm)

Carrier gas: He 5.5, 1 mL / min constant debit.

Oven temperature:

	Rate °C/min	Value °C	Hold Time min	Run Time min
Initial		60	2	2
Ramp 1	10	140	0	10
Ramp 2	4	240	0	55
Ramp 3	10	300	4	49

Total run time : 49 min

MS Conditions: EI ion source temperature : 230°C, full scan mode (m/z 50-800)

Quad parameters

EI ion source temperature: 230°C

Quadrapole Temperature:150°C

Electron energy: 70eV

ToF parameters

Acquisition 2GHzEDR with N2 (1.5mL/min)

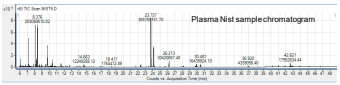
Acq rate: 5 spectra/s

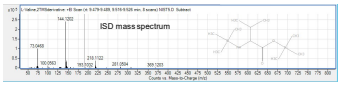
Acq time: 200ms/spectrum

Transients by spectrum: 2712

Limits for average PPM error: 3.0 and maximun error: 8.0

Bioinformatic tools : Available workflow to process GS-MS data





Raw data files are processed and converted to mzdata format using the Agilent MassHunter software.
Data are processed under the Galaxy web-based platform Worflow4metabolomics, using MetaMS or XCMS packages :

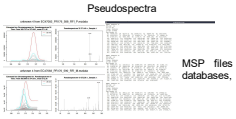
Two setting modes exist if the user need to optimize extraction parameters

After extraction step, a data matrix file is generated and can be used for statistical analysis. Intensities are the sum of intensities of all ions from the pseudospectrum (=unknown x) :

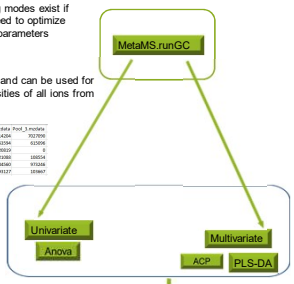
Name	Blanc_TMS_1.mzdata	Blanc_TMS_2.mzdata	Blanc_TMS_3.mzdata	Pool_1.mzdata	Pool_2.mzdata	Pool_3.mzdata
Unknown 226	0	0	0	5567567	8414264	7027896
Unknown 325	0	0	0	0	582594	615006
Unknown 270	0	0	0	200658	520010	0
Unknown 348	0	0	0	0	121000	160034
Unknown 254	0	0	0	893227	985460	973266
Unknown 354	0	0	0	0	91127	101667

A peakspectra.msp file is produced that contains all the spectra of the detected compounds in MSP format.

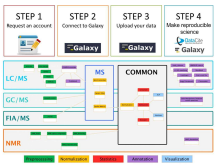
Pseudospectra



MSP files are used to query in-house databases, and NIST, or Golm databases.



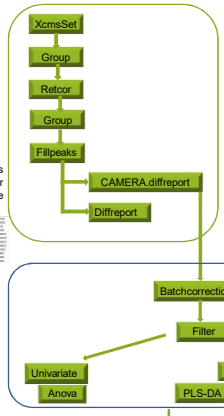
Workflows : Workflow4Metabolomics 3.0 Galaxy online infrastructure



Extraction method for peak detection :

Matched filter (R= 6000)

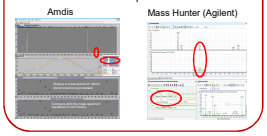
Data matrix file is generated: it represents for each ion, the value of the intensity in each sample



Preprocessing

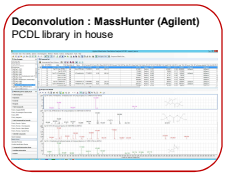
Statistics

GC-MS : deconvolution strategy to identify compounds



Golm Metabolome Database (http://gmd.mpimp-golm.mpg.de/)

NIST (http://www.nist.gov/srd/mslist.htm)



Deconvolution : MassHunter (Agilent)
PCDL library in house

MassBank (http://www.massbank.jp/)

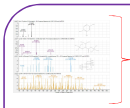
Annotations

Complementary analysis in low resolution are necessary to elucidate chemical structure of unknown compounds :

-> CI ionization mode

-> MS² on QqQ instrument

Quattro Micro GC Waters



Accurate mass measurement

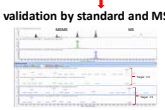
Determination of elemental formula

Database queries
Reduction of the hit numbers because of higher accuracy and resolution

Hypothesis validation by standard and MS² analysis

GC-QToF 7200 Agilent

Cx Hy Oz



Structural identification

Development of new tools

Data extraction :

To process high resolution data using MetaMS package it is necessary to optimize extraction parameters. Therefore, a tool was developed in the lab to realize this step. Several parameters can be considered (e.g : FWHM, similarity_threshold,…) one by one or in combination.

Identification

Reference mass spectra are acquired in high resolution in order to implement Peak Forest database.

NIST plasma samples are also analyzed by GC-QToF (MS, MS²) to annotate reference compounds in the matrix.

Conclusion and perspectives

Annotated spectra of reference compounds and biological matrices will be implemented in the metaboHUB national reference database (Peakforest).
Accurate mass measurements, as well as the development of new tools for data processing should allow solving the actual bottleneck in biomarker discovery, concerning the identification of metabolites.

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