Establishing a GC-MS Based Metabolomics Platform

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Metabolomics Workflow

Platform support

The platform offers several options for sample preparation, extraction, injection, separation and detection of metabolites as well as tools for data processing.

Biological Question

Experiment design

Experiment, Sampling

Targeted analysis to control progress of experiment

Sample Preparation

Sample homogenisation with cryomill or blender

Lyophilisation

Liquid extraction (experiment design for optimal conditions)

Storage at -80℃

GC-MS Analysis

Injection: liquid, HS, SPME (for highly automated sample extraction and injection)

Different sample matrices and metabolites of interest require optimisation of SPME parameters for every new experiment to maximise the number of extracted metabolites. D-optimal design [1] provides optimal SPME parameters with a rather low number of injections.

Separation: polar, apolar, midpolar columns

Instruments: Thermo Trace GC Ultra TSQ Quantum XLS (untargeted analysis)
Thermo Trace GC Ultra TSQ Quantum GC (targeted analysis, MRM)
Waters GCT Premier TOF accurate mass (confirmation analysis)

Instrument and method performance check:
- Regularly injection of quality control (QC) samples and standard mixtures
- Daily check of raw chromatograms by operator
- Check several samples or whole sequence via PCA (fig. 5) on basis of XCMS [2] extracted features (web based interface, XCMS pipeline, fig. 3)

Data Processing

Convert raw files to CDF

All data processing steps are based on R [3] scripts (partly developed in-house)

Peak picking by XCMS [2]

Reconstruct mass spectra with modified CAMERA [4]

Annotation: compare mass spectra with in-house and/or commercial spectra libraries

Identification: compare RT(RI) with authentic standard

In-house mass spectra library:
- about 350 standards yet, every spectra checked manually
- Spectra for TOF and Quadrupol
- RT/RI for WAX and RTX-200 column
- Library entries linked to CAS-Nr., InChI-key, Pubchem-ID, etc.

Chemometrics, Statistics

Biostatistics
Univariate: e.g. t-test
Multivariate: e.g. PCA

Example: Grape

Explore the volatile metabolic space of grapevine berries by investigation of about 120 *Vitis* varieties

Collect grape berry samples for 4 vintages from the experimental vineyard

Ground berries with a blender under cooling with lq. N_2

Prior to analysis add:
- preservatives (sodium azide, citric acid, ascorbic acid) to minimise enzymatic and oxidative reactions
- $MgSO_4$ to decrease solubility of metabolites
- Water, labelled IS, stir bar

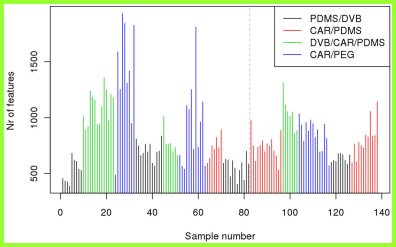

Fig. 2 Bar plot showing number of features from every single injection representing different combinations of the tested parameters (from [1]).

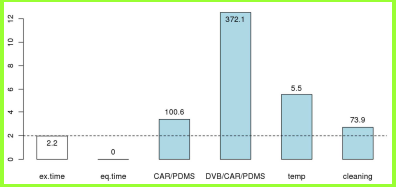

Fig. 1 Significance of the coefficients in the linear model of the D-optimal design. Filled bars correspond to significant (0.05 level) coefficients (from [1]).

Experimental design:
- Generation of all possible experiments: 768
- D-optimal subset: 32
- Adding experiments and replicates: 138 injections

Tested parameters:
- Equilibration time: 0-35 min
- Extraction time: 20-50 min
- Temperature: 30, 45, 60℃
- SPME fibre type
- Cleaning of instrument

Used parameters:
- Equilibration time: 20 min
- Extraction: 30 min
- Temperature: 60℃
- Fibre: 2 cm DVB/CAR/PDMS

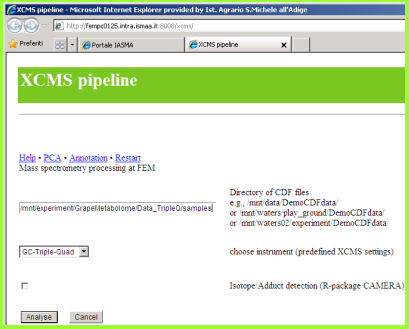

Fig. 3 Screenshot of the web based interface (XCMS pipeline) for QC.

Quality control on basis of XCMS extracted features is a simple and easy to use tool to check quickly if a sequence in general is fine or some samples have to be repeated (e.g. those where the IS has not been added, see fig 5).

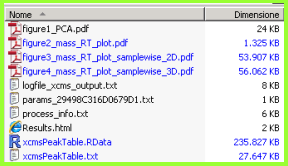

Fig. 4 Resulting files of the XCMS processing.

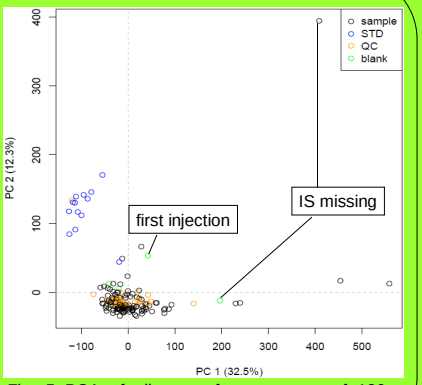

Fig. 5 PCA of all runs of a sequence of 120 samples (160 injections).

Table 1 Identified metabolites of a Gewürztraminer sample.

Hexanal
beta-Myrcene
E-2-Hexenal
2-Pentylfuran
p-Cymene
3-Octanone
2,2,6-Trimethylcyclohexane-1-one
E-2-Heptenal
6-Methyl-5-hepten-2-one
1-Hexanol
Z-3-Hexenol
EE-2,4-Hexadienal
2,4-Hexadienal isomer
E-2-Hexenol
E-2-Octenal
Octanoic acid ethyl ester
1-Octen-3-ol
EE-2,4-Heptadienal
Benzaldehyde
E-2-Nonenal
beta-Cyclocitral
Decanoic acid ethyl ester
Beta-Citronellol
Methyl salicylate
Nerol
Geraniol
Hexanoic acid
Benzyl alcohol
2-Phenylethanol
beta-Ionone
2-Phenoxyethanol
Ethyl anthranilate
2,4-bis(dimethylethyl)-phenol
Benzyl benzoate

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