ELECTRONIC SUPPLEMENTARY INFORMATIONS

Sensitive, highly resolved, and quantitative ^{1}H-^{13}C NMR data in one go for tracking metabolites in vegetal extracts

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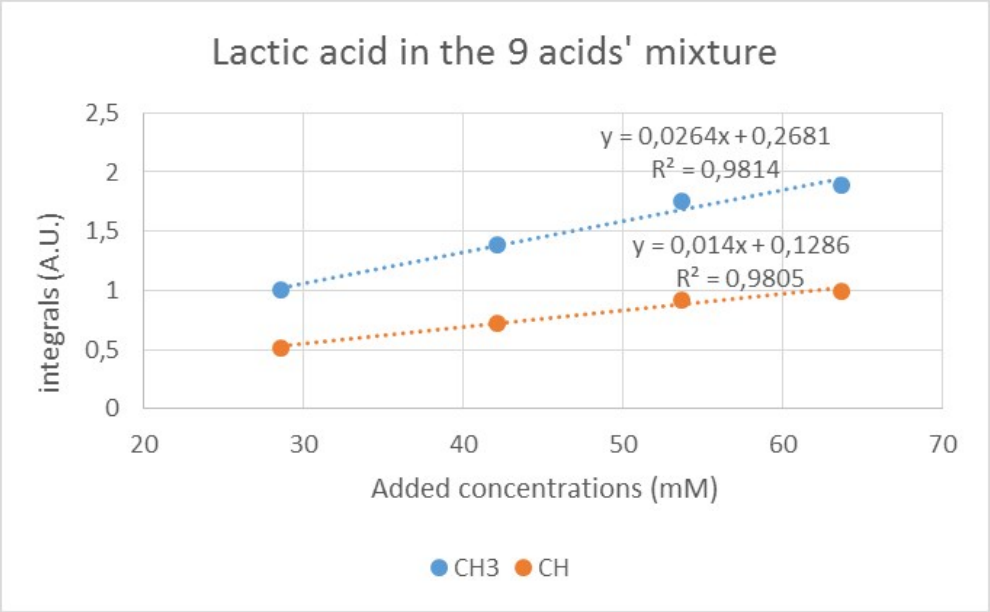
I. Standard addition method to access concentrations

1D ^{1}H NMR was used to obtain integrals of different standards by changing their concentration. The concentration before addition or the initial concentration could be obtained by plotting the integrals of a given signal regarding the added concentration values. The initial concentration of the desired standard to quantify shall be the x-intercept equal to the y-intercept divided by the slope of the linear regression curve [1] (see below):

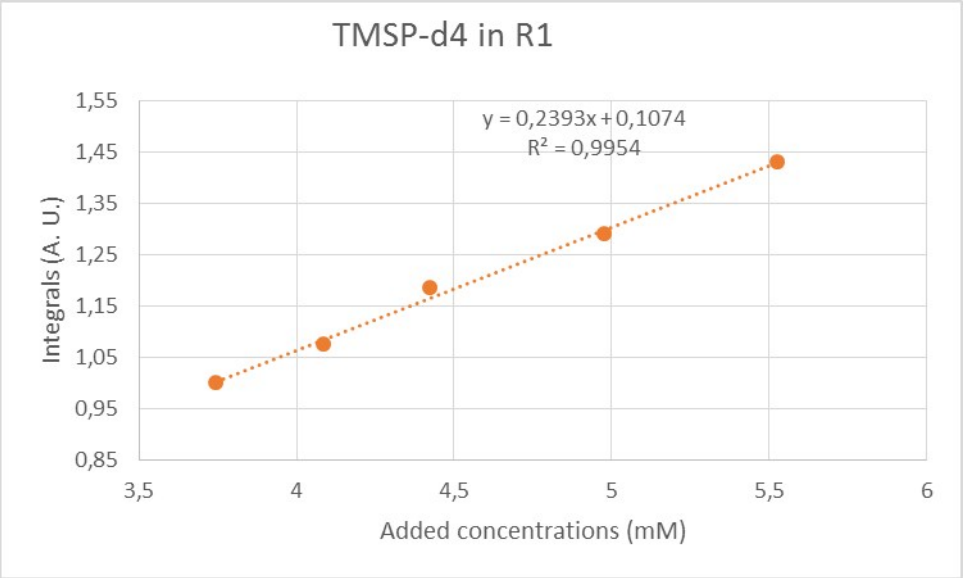
Lactic acid in the model sample of acids

[lactic]=(0.268/0.026 + 0.128/0.014)/2=9.7 mM

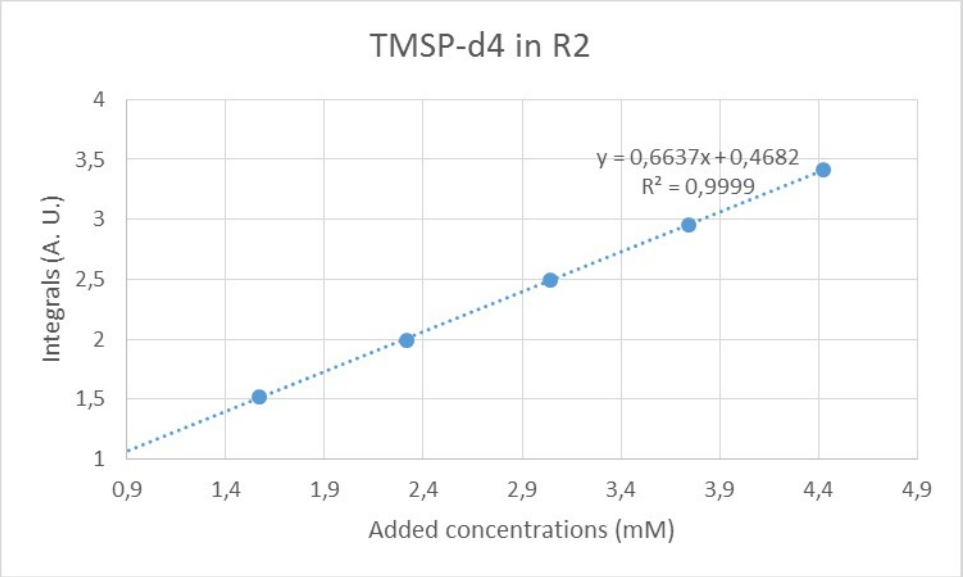


TMSP-d4 in the R1 and R2 Arabidopsis Thaliana samples

In R1: [TMSP-d4]=0.107/0.239=0.45 mM



In R2: [TMSP-d4]=0.468/0.664=0.70 mM



II. Concentration calculation with the HSQC0 experiment

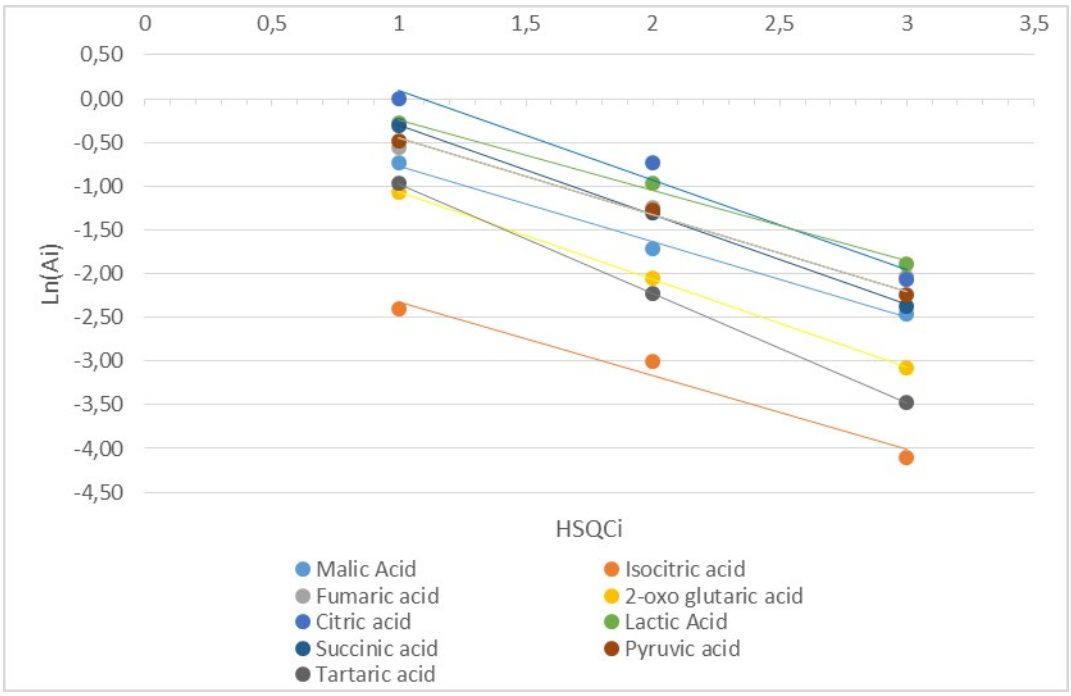
The gradient selective $HSQC_0$ was used to access the peak volume in the $HSQC_i$ with i=1, 2, 3 through linear regression extrapolation of:

$Ln(Ai,n)=Ln(2A_0)+i.Ln(f_{An}.\ 0.5)$ [2,3,4]

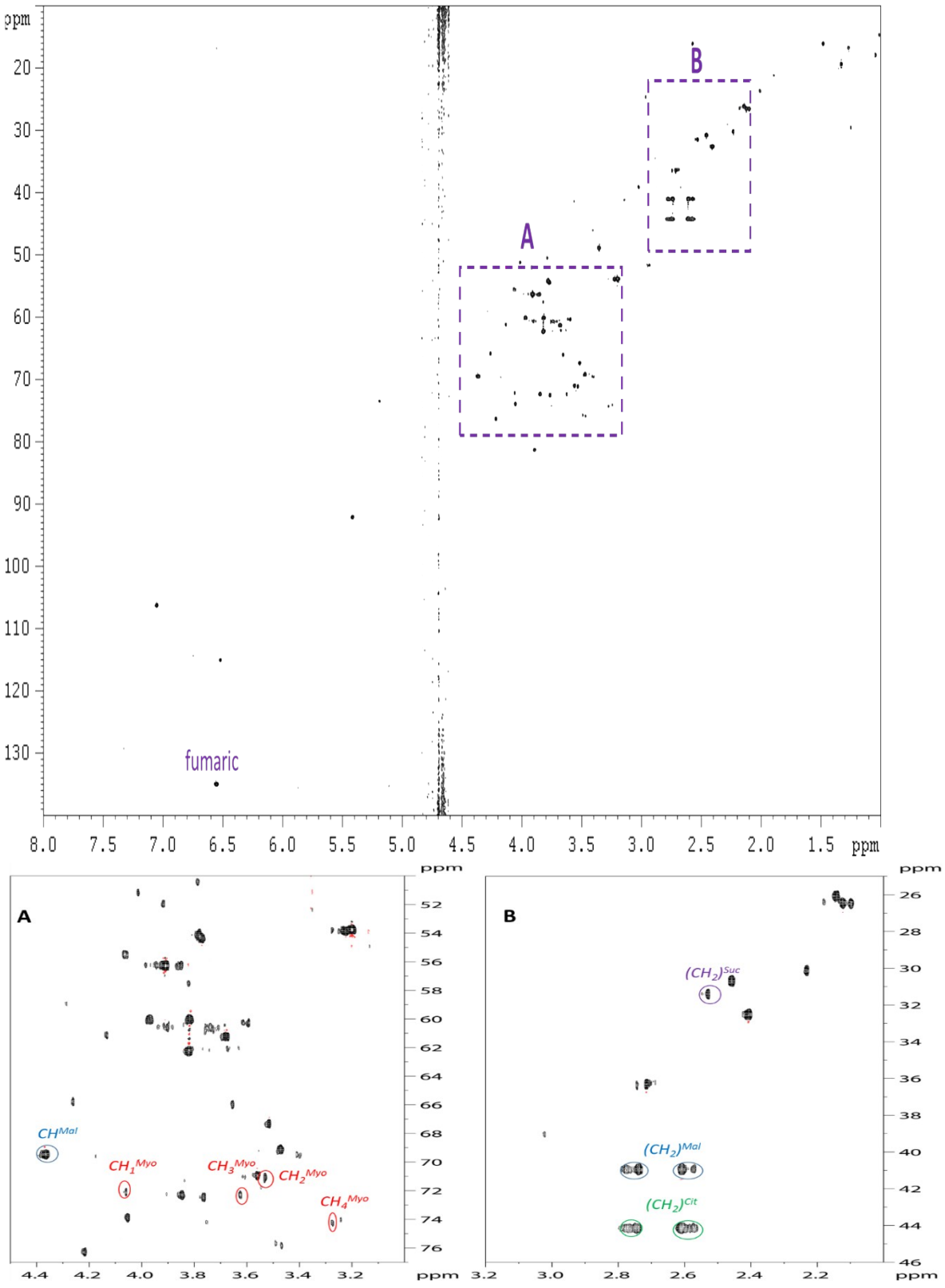
With Ai,n is the integrated peak intensity for the peak n of the $HSQC_i$, A_0 is the virtual integrate peak intensity of the virtual $HSQC_0$ and f_{An} is the amplitude attenuation factor for the peak n

	Ln(A1)	Ln(A2)	Ln(A3)	A0 normalized	[conc] mM
Malic Acid	-0.73	-1.72	-2.46	0.27	9.2
Isocitric acid	-2.41	-3.00	-4.10	0.18	6.1
Fumaric acid	-0.56	-1.25	-2.04	0.30	10.2
2-oxo glutaric acid	-1,07	-2.05	-3.07	0.27	8.9
Citric acid	0.00	-0.73	-2.06	0.39	10.4
Lactic Acid	-0.29	-0.96	-1.89	0.29	9.70
Succinic acid	-0.31	-1.30	-2.37	0.26	8.7
Pyruvic acid	-0.48	-1.27	-2.24	0.25	8.5
Tartaric acid	-0.96	-2.23	-3.47	0.33	11.1

Ln(Ai,n) values were averaged over three series of experiments


0
0,5
1
1,5
2
2,5
3
3,5
0,50
0,00
-0,50
-1,00
-1,50
-2,00
-2,50
-3,00
-3,50
-4,00
-4,50
Ln(Ai)
HSQCi
Malic Acid
Isocitric acid
Fumaric acid
2-oxo glutaric acid
Citric acid
Lactic Acid
Succinic acid
Pyruvic acid
Tartaric acid

III. QUIPU HSQC map on R1 leave extract



^{1}H-^{13}C QUIPU HSQC (full map on the top) on the R1 extract of the wild type Arabidopsis Thaliana's leaves. Selected regions (A,B bottom) Blue, red, purple, and green circles focus on cross peaks of malic acid, myoinositol, succinic, and citric acids respectively

IV. RMSD for concentrations determined from QUIPU HSQCs on different rosettes (see Figure 6)

RMSD	R1	R2
Myo	0.01	0.05
Mal	0.05	1.28
Cit	0.09	2.41
Fum	0.08	0.38
Suc	0.01	0.05

V. Samples preparation

Myoinositol:

A 100 mM sample was prepared in H_20

9 acids mixture:

Malic, isocitric, citric, pyruvic, succinic, tartaric, 2-oxo-glutaric, lactic and fumaric acids were dissolved in H_2O with ~10 mM concentration (see Figure 4). For each acid a solution of 100mM was prepared. Then, 100µl of each acid + 100µl of H_2O were mixed together to reach 10mM for each acid. The NMR sample was composed on 590 µL of the 9 acids' mixture with 10 µL of D_2O containing 0.75% w/w TMSP-d_4.

Arabidopsis Thaliana leaves:

Protocol used for extracting metabolites [5,6]

500mg of fresh leaves were pooled and freezed under liquid nitrogen to quench intracellular enzymatic activity. This tissue was ground thoroughly in a mortar in liquid N_2. Then 2ml of solvent (H_2O 20%: MeOH 80%) were added to extract the polar molecules. Tissue suspension were transferred to 2-mL eppendorf tubes, and centrifuged at 10 000 g under 4°C for 15 min. Supernatants were transferred to fresh tubes and centrifuged again. Aliquots of 1.6 mL (for R1 and R2) were spun-dried under vacuum and stored at 80°C until analysis. For the analysis, the resulting dried material was re-dissolved in 590 µL D_2O with 10 µL of D_2O containing 0.75% w/w TMSP-d_4.

VI. NMR experimental details

All experimental data were obtained using a Bruker 400 MHz AVANCE III HD equipped with a Prodigy cold BBO $^1H/X$ probe with a z gradient. The maximum gradient is 50 G.cm^{-1}. The temperature was stabilized at 298 K. For each HSQC experiment a GARP-1 heteronuclear decoupling of 4.2 kHz was used on ^{13}C.

Myoinositol:

The spectra on Figure 3 were acquired using a 100 mM myoinositol sample in H_2O. For Quantitative Q-HSQC, 8 scans per increment have been necessary for each 8 experiment-in the indirect dimension. $^1J_{CH}$ compensation was carried out with 8 INEPTs 6 with short delays

delays Δ=1.47 ms and 2 with a longer delay Δ=2.96 ms. The recycle delay was set to 15 s (5 time the maximum T_1=5s) for ensuring a complete longitudinal relaxation process for quantitative data. Under these conditions, each experiment required 18 min.
For the QUIPU HSQC, the pure shifting of the acquisition was performed with 20 chunks of 30 ms and BIRD/180° elements have been used to ensure no J_{HH} evolution during the acquisition time.

9 acids mixture:

The spectra on Figure 4 were acquired using 9 acids with about 10mM concentration: citric, isocitric, 2-oxo glutaric, succinic, pyruvic, tartaric, lactic, malic, and fumaric in H_2O. For Quantitative Q-HSQC, 8 scans per increment have been necessary for each 8 experiment-in the indirect dimension. $^1J_{CH}$ compensation was carried out with 8 INEPTs 6 with short delays delays Δ=1.47 ms and 2 with a longer delay Δ=2.96 ms. The recycle delay was set to 24 s (5 time the maximum T_1) for ensuring a complete return to the Boltzman equilibrium for quantitativity. Under these conditions, each experiment required 4h. Matrices of 6k x 44 have been zero filled up to 8k x 256.
For the QUIPU HSQC, the pure shifting of the acquisition was performed with 20 chunks of 30 ms and BIRD/180° elements for 600 ms of acquisition time.
The HSQC1 HSQC2 HSQC3 maps have been recorded with d1=24s ns=24, each matrix of 6k x 44, and aq=0.6s.

Arabidopsis thaliana's leaves:

The spectra on Figure 5, ESI-III were acquired on R1 and R2 extracts in D_2O (see V). For Quantitative Q-HSQC, 40 scans per increment have been necessary for each 8 experiment-in the indirect dimension. $^1J_{CH}$ compensation was carried out with 8 INEPTs 6 with short delays delays Δ=1.47 ms and 2 with a longer delay Δ=2.96 ms. The recycle delay was set to 20 s (5 time the maximum T_1) for ensuring quantitative data. Under these conditions, each experiment required 3 days. Matrices of 6k x 400 have been zero filled up to 8k x 1024.
For the QUIPU HSQC, the pure shifting of the acquisition was performed with 20 chunks of 30 ms and BIRD/180° elements for 600 ms of acquisition time.

VII. References

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