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Multiplatform Plant Metabolomics Analysis Protocol for Arabidopsis thaliana

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We use this protocol and it's working

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Abstract

In the pursuit of comprehensive metabolomic profiling, a multiplatform analysis protocol has been developed for *Arabidopsis thaliana*, utilizing an array of analytical techniques to quantify a diverse set of intracellular metabolites. This protocol encompasses methods for the extraction and purification of metabolites, followed by their analysis using high-performance liquid chromatography (HPLC) for amino acids, liquid chromatography-tandem mass spectrometry (LC-MS/MS) for central carbon metabolism, and gas chromatography-tandem mass spectrometry (GC-MS/MS) for open profiling of amino acids, organic acids, sugars, and fatty acids. Each method is meticulously designed to ensure precise quantification and identification of metabolites critical for understanding the metabolic networks in *Arabidopsis thaliana*. This protocol not only outlines the steps for metabolite extraction and analysis but also details the preparation of reagents and solutions, instrumental settings, and the analytical conditions required for optimal detection and quantification. The integration of these platforms provides a robust framework for metabolic studies, contributing significantly to the field of plant metabolomics by enabling detailed metabolic profiling with high accuracy and reproducibility.

Image Attribution

Image made using Biorender (Agreement number: PO26Z2LCDS)

Materials

1. Extraction and Purification of Intracellular Metabolites

1.1 Materials and Supplies

- 0.5 mm glass beads
- Dry ice
- 2 ml centrifuge tubes
- 1 ml clear glass vials
- 300 µl glass inserts
- 2 ml reinforced tubes with screw caps (Scientific Specialties Inc. Cat No. 2320-00 and 2001-00) for bead beating

1.2 Equipment

- Vortex mixer
- Analytical balance
- Pipettes (1 ml, 100 µL)
- Centrifuge (with cooling capacity to 4°C)
- Mini bead beater (Omni BR24 bead beater)
- Vacuum concentrator
- Freeze drier

1.3 Chemicals and Reagents

- **Methanol:** Solvent for extraction and purification of metabolites.
- **Ethanol:** Used for prechilling equipment.
- **Millipore filtered distilled water (MQ water):** Provides a pure water source for extraction.
- **100% Acetonitrile (LC grade):** Used for reconstitution of samples before analysis.
- **Chloroform:** Solvent for biphasic separation to remove lipids and proteins.
- **Azidothymidine (AZT):** Internal standard for quantification.

2. HPLC — Amino Acid Analysis

2.1 Materials and supplies

Consumables

- 1.5 mL centrifuge tubes
- 1.8 mL HPLC glass vials
- HPLC glass insert
- Agilent Zorbax Extend C-18 column (3.5 µm, 4.6 × 150 mm, Agilent PN: 763953-902)
- Guard column (SecurityGuard Gemini C18, Phenomenex PN: AJO-7597)

For sample digestion:

- Digestion vials
- 0.2 µM syringe filters
- Syringe with Luer lock system

Equipment

- HPLC System with FLD
- Vortex mixer
- Centrifuge (with cooling capacity to 4°C)
- pH meter
- Filtering and degassing apparatus with 0.45 µm nylon and PTFE membrane filters
- Magnetic stirrer
- Oven, incubator or heat block (with heating capacity of 110°C)
- Fume cupboard

2.2 Chemicals and Reagents

Sample digestion:

- High purity water (18.2 MΩ.cm at 25 °C)
- 6M hydrochloric acid
- Phenol
- 6M Sodium hydroxide
- Nitrogen gas
- Lithium hydroxide monohydrate (Sigma 62528-50G)

Derivatisation and HPLC analysis:

- Internal standard - α-aminobutyric acid, norvaline and sarcosine 500 µM each
- External standard - amino acid standards 500 µM each and containing 250 µM of internal standards.
- Reducing agent - 0.5% (v/v) 3-mercaptopropionic acid (Sigma M5801) in borate buffer (0.4 N, pH 10.2, Agilent PN: 5061-3339)
- Alkylating agent - 120 mM iodoacetic acid (Sigma, I4386) in 140 mM NaOH
- OPA reagent - 10 mg o-phthalaldehyde/mL in 3-mercaptopropionic acid, (Agilent PN: 5061-3335)
- Fmoc reagent - 2.5 mg 9-fluorenylmethyl chloroformate/mL in acetonitrile (Agilent PN:5061-3337)
- Sodium phosphate dibasic (Na₂HPO₄)
- Sodium azide (NaN₃)
- Hydrochloric acid
- Acetonitrile (HPLC grade)
- Methanol (HPLC grade)

2.3 Preparation of solutions and reagents

External standards (calibration points)

Stock solution of external standards is prepared with 500 μM concentration (L1) of each amino acid analysed, containing 250 μM internal standard (ABU, NVal, Sar). The solution is aliquoted at 50 μL in PCR tubes and stored at -80°C freezer until use. The following concentrations are usually analysed and used as calibration points.

	stan dard	Concentratio n (μM)
	L1	500
	L2	250
	L3	125
	L7	7.81
	L8	3.91
	L9	1.95

Mobile phases

Mobile phase A (10x solution): 400 mM Na_2HPO_4 , 0.2% NaN_3 .

Adjust to pH 7.8 using HCl. Vacuum filter. Store in 4°C and dilute to 1x with MQ water before use. Note: precipitates may form during storage. Mix well or apply heat to resuspend in solution.

Mobile phase B: 45% Acetonitrile, 45% Methanol, 10% water

Derivatisation reagents

Borate Solution: Prepare Borate solution by mixing 995 μL Borate buffer and 5 μL 3-mercaptopropionic acid.

FMOC solution: by mixing 30 μL of 10x stock FMOC reagent (made in—house) and 270 μL acetonitrile.

3. LC-MS/MS — Central Carbon Metabolism (CCM)

3.1 Materials

- 2 mL HPLC glass screw cap vials with inserts
- Pipettes (5 mL, 1 mL, 200 μL)

3.2 Chemicals and Reagents

- Azidothymidine (AZT) as internal standard
- Glacial acetic acid
- Tritbutylamine (TBA)
- Millipore filtered distilled water (MQ water)
- External standards (calibration points): Individual chemicals are prepared in 200 μ M concentration with water and stored at -80°C freezer. Stock solutions are serially diluted from 200 μ M to 1.5nM and added with 5 μ M AZT.
- Mobile Phases A: 7.5mM Tritbutylamine, acetic acid to pH 4.95.
- Mobile Phase B: 100% Acetonitrile
- Rinse solution – 10% isopropanol

4. GC-MS/MS — Open profiling—amino acids, organic acids, sugars, fatty acids

4.1 Materials

- 1.5 mL centrifuge tubes
- 1.8 mL HPLC glass vials
- HPLC glass insert
- Agilent DB-5 column (30m, 0.25mm x 1 μ m, Agilent PN: 122-5033)

4.2 Chemicals and Reagents

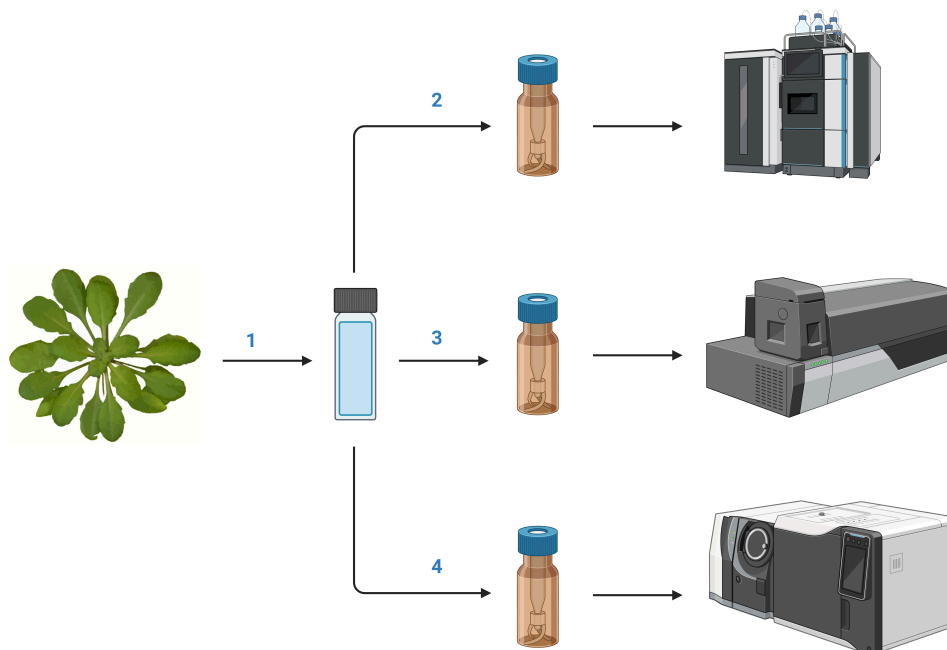
- Methoxyamine HCl (Merck #89803)
- Pyridine (Merck # 360570)
- BSTFA +1%TMCS (Macharey Nagel)
- 13C- D-sorbitol (Merck #605514)

4.3 Equipment

- Vortex mixer
- Analytical balance
- Pipettes (1 ml, 100 μ L)
- Centrifuge (with cooling capacity to 4°C)
- Vacuum concentrator

1. Extraction and Purification of Intracellular Metabolites

- 1 Weigh approximately 25 mg of freeze-dried *Arabidopsis thaliana* tissues into bead beating tubes. Record the exact weights. Keep samples frozen.  0 °C
- 2 Add approximately 200 µl of 0.5 mm silica glass beads to each sample. Prechill the bead beater (OMNI Bead Ruptor Elite NE486LUA) to  -1 °C with ethanol and dry ice.
- 3 Extract tissue samples in the bead beater using the following settings:
 - Speed: 7.00 m/s
 - Pulses: 3 × 45 sec
 - Interval: 30 sec between pulses
- 4 Add 1 ml methanol to the ground samples. Vortex mix.
- 5 Add 0.4 ml chloroform to the samples. Vortex mix. Sonicate in an ice bath for 10 min. ALiquot 500 µl of samples for GC-MS open profiling (Step 23).
- 6 Add 500 µl MQ water with 500 nM AZT. Vortex mix.
- 7 Centrifuge at 16,000 RCF for 5 minutes at  4 °C . Transfer approximately 1 ml of the aqueous layer to new 2 ml Eppendorf tubes, avoiding the interface layer containing proteins.
- 8 Evaporate methanol in the samples using a vacuum concentrator until about 400 µl of liquid (water fraction) remains. This step ensures low levels of methanol in preparation for freeze drying.
- 9 Freeze dry the samples overnight.
- 10 Reconstitute in 200 µl of 2% acetonitrile solution (5x concentrated).
- 11 Let stand for 10 minutes on ice. Centrifuge at 16,000 RCF at  4 °C for 5 minutes, then transfer 200 µl into HPLC glass vial insert. Samples can be used directly for LCMS and HPLC analysis, or stored at  -80 °C freezer.



Schematic diagram of Multiplatform Plant Metabolomics Analysis Protocol for *Arabidopsis thaliana*

1. Extraction and Purification of Intracellular Metabolites
2. HPLC – Amino Acid Analysis
3. LC-MS/MS – Central Carbon Metabolism (CCM)
4. GC-MS/MS – Open profiling—amino acids, organic acids, sugars, fatty acids

2. HPLC — Amino Acid Analysis

12 25 μL of crude solution of samples are added to 25 μL of 1 mM internal standards.

13 Instrumentation

Instrument: Thermo Scientific Vanquish Core UHPLC with FLD detector

Column: Agilent Zorbax Extend C-18 column 3.5 μm , 4.6 $\times$ 150 mm (PN: 763953-902)

Guard Column: Phenomenex SecurityGuard Gemini C18 (PN: AJO-7597)

Detector FLD 1: OPA-derivatised amino acids detected at 340_{ex} and 450_{em} nm

Detector FLD 2: FMOC-derivatised amino acids detected at 260_{ex} and 325_{em} nm



Thermo Scientific Vanquish Core UHPLC with FLD detector

14 Derivatisation

Aliquot FMOC reagent, OPA reagent, Borate buffer, Iodoacetic acid and Mobile phase A into HPLC vials. Take note of the position in the HPLC autosampler. Derivatisation is performed as previously published [Valgepea et al 2017]. The following steps are programmed in a high-performance autosampler.

- 14.1 1 μL of sample (which has been diluted 1:1 with internal standard), is added into 3 μL of 0.5% (v/v) 3-mercaptopropionic acid (Sigma M5801) in borate buffer (0.4 N, pH 10.2, Agilent PN: 5061-3339), mixed and incubated for 20 s at 4°C , to reduce free cystines.

- 14.2 To alkylate reduced cysteines, 1 μL of 120 mM iodoacetic acid (Sigma, I4386) in 140 mM NaOH is added, mixed and incubated for 20 s at 4°C .
- 14.3 1.5 μL of OPA reagent is then added to derivatise primary amino acids. The reaction was mixed and incubated for 20s at 4°C .
- 14.4 FMOC reagent (1 μL) is added, mixed and incubated for 20 s at 4°C to derivatise the secondary amino acids.
- 14.5 The pH is then lowered by adding 50 μL of Mobile phase A (pH 7). The whole 57.5 μL are then injected to the UHPLC.

15 UHPLC parameters

The UHPLC gradient:

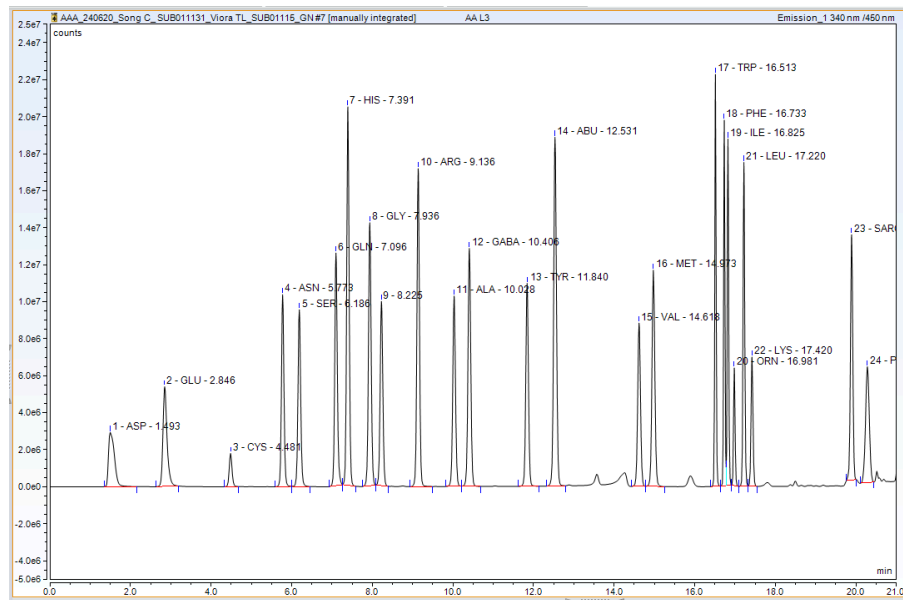
A	B
Mobile phase B concentration (%)	Time (min)
2 – 30%	0 – 14
30 – 25%	14.1 – 15
40 – 45%	15.1 – 18
50 – 60%	18.1 – 20
100%	20.1 – 25
2%	25.1 – 27

Column Temperature: 37°C

Flow rate: 1.8 mL/min

- 16 Derivatised amino acids are monitored using a fluorescence detector. OPA-derivatised amino acids were detected at 340_{ex} and 450_{em} nm from 1-18 min, and FMOC-derivatised amino acids at 260_{ex} and 325_{em} nm from 18-21 min.

- 17 Quantifications are based on standard curves derived from serial dilutions of a mixed amino acid standard. The upper and lower limits of quantification are 1000 and 1.95 μM , respectively. Chromatograms are integrated using Chromeleon software.



Chromatogram

3. LC-MS/MS — Central Carbon Metabolism (CCM)

- 18 Data acquisition was performed using negative ionization mode, and all analyses were executed on a Shimadzu 8060 LC-MS/MS system.

Instrument: Shimadzu 8060 LC MS-MS

Column: Phenomenex Gemini NX-C18 $3\mu\text{m}$ x 150mm x 2mm (Part No. 00F-4453-B0)

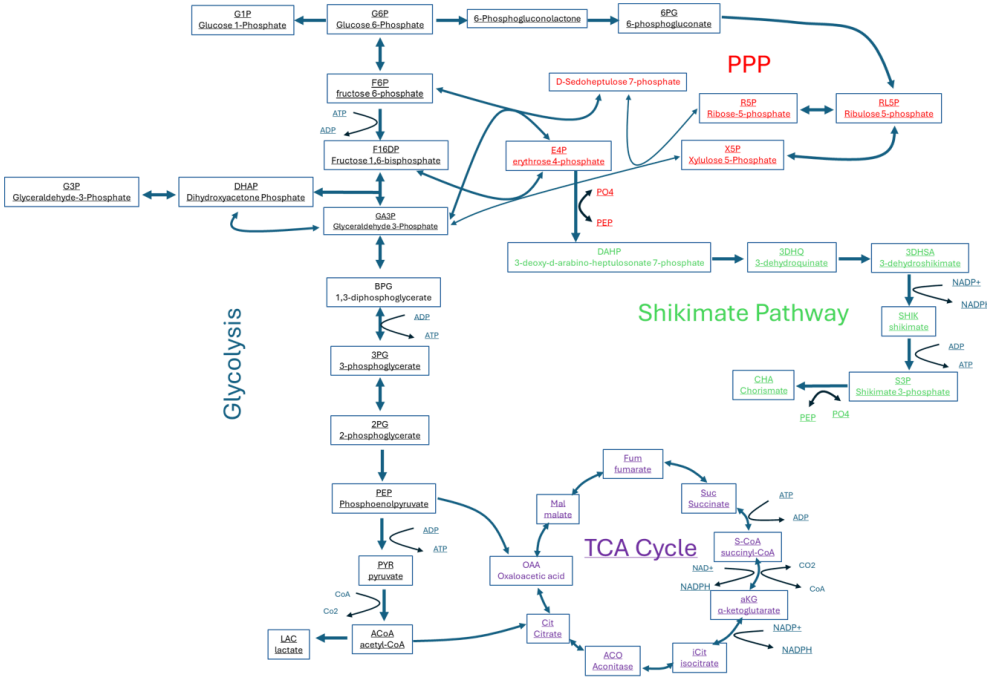
fitted with

Phenomenex SecurityGuard column Gemini-NX C18 $4 \times 2.0\text{mm}$ ID (AJ0-8367).



Shimadzu 8060 LC MS-MS System

19 CCM method compound list



The Central Carbohydrate Metabolic Network.
Metabolites targeted in LC-MS/MS in this protocol are underlined.

Multiple reaction monitoring (MRM) transitions (Q1/Q3)
List for identification and quantification of CCMs.

A	B	C	D	E	F
	Q1	Quantifier ion	Qualifier ion	Precursor CE	
Compound Name	m/z (1)	m/z(2)	Ref.(1) m/z(2)	Target Collision Energy	RT (min)
2-Keto-3-Deoxy-6-Phosphogluconate	257. 2	97.05	78.8	16.9	25.79 7
3- Phospho-D-Glycerate	185	96.95	78.95	15.6	24.75 1
3-Dehydroquinic Acid	189. 05	171.2	109.05	13.2	6.801
3-Dehydroshikimic Acid	171. 05	109.05	127.1	19.7	8.627
3-Hydroxybutyrate	103. 3	59	41	13.3	9.295



A	B	C	D	E	F
3-Hydroxybutyrate Coenzyme A	852. .15	408.05	158.85	40.2	36.5
6-Phosphogluconic Acid	275. 1	96.9	78.95	17	24.39
α -Ketoglutarate	145. 2	101	57.1	11.9	23.34 9
Acetoacetyl Coenzyme A	850. .15	766.1	158.85	28.9	37
Acetolactate	131. 25	87.15	42.05	9.7	16.64 6
Acetyl Coenzyme A	807. 9	408.1	461.1	37.2	38.37 2
Acetylphosphate	139. 2	78.8	63	20.8	20.58 9
Aconitic Acid	173. 1	85.05	129.15	13.2	25.98 2
Adenosine Diphosphate	426	134.1	159.1	23.4	25.84
Adenosine Diphosphate	741. 95	620	408.05	17.6	24.71 1
Adenosine Monophosphate	346 .05	79	96.95	24.7	17.66 2
Adenosine Triphosphate	505 .9	158.95	408.05	30.6	35.58 5
Adipic Acid	145. 25	101.05	83.15	15	21.07 5
Anthranilic Acid	136. 25	92.1	65	16.6	22.28 3
Azidothymidine (Internal standard)	266 .1	223.25	42	11.3	15.88 7
Citrate	191	87.05	111.05	18.2	25.43 7
Coenzyme A	766	408.05	419.1	36.2	37.60 4
Creatine Phosphate	210. 05	79	97	21.7	21.911
Cytidine Monophosphate	322 .15	96.95	79	21.6	11.48 5

A	B	C	D	E	F
Cytidine Triphosphate	482	159.1	384.1	30.1	34.23 ₅
D-Erythrose 4-Phosphate	199. ₁	96.95	79	10.3	12.12 ₉
Dihydroxyacetone Phosphate	169. ₂	97	79	10.7	12.73 ₄
D-Ribose 5-Phosphate	229. ₁	97	79	13.8	8.969
D-Ribulose 5-Phosphate	229. ₁	97	79	11.6	11.163
D-Xylulose 5-Phosphate	229. ₁	97	79	11.6	10.80 ₆
Flavin Adenine Dinucleotide	784. ₁₅	437.1	346.1	28.4	30.4 ₄
Flavin Adenine Mononucleotide	454. ₉	97	78.85	26.4	24.28 ₁
Fructose 1,6-Bisphosphate	339. ₁	96.95	79	22	25.16 ₁
Fructose 6-Phosphate	259	96.95	79.05	16.8	9.345
Fumarate	115. ₂	71.05	27	11.4	24.3
Glucose 1-Phosphate	259	79.05	241.15	23.1	9.93
Glucose 6-Phosphate	259. ₁	96.9	78.9	18.1	8.195
Glyceraldehyde 3-Phosphate	169. ₁	96.9	78.9	10	9.075
Glycerol 3-Phosphate	171.1	79.05	96.85	23.3	9.695
Glycolic Acid	75.3	47.05	44.95	14.2	6.514
Guanosine Diphosphate	442	344.1	159.05	20.5	24.96
Guanosine Monophosphate	362. ₀₅	79.05	211.15	24.5	15.23 ₄
Guanosine Triphosphate	522	159.1	424	33.4	35.01 ₉
Isocitrate	191.1	73	173.2	21.9	25.63 ₃

A	B	C	D	E	F
Lactate	89. 3	43	45.05	14.4	8.684
Malate	133. 2	115.1	71	16	22.11 4
Malonyl Coenzyme A	852	808.1	408	26.1	20.1
Nicotinamide Adenine Dinucleotide (Oxidised)	662 .1	540.15	273.05	15.9	14.16 8
Nicotinamide Adenine Dinucleotide (Reduced)	664	408.1	397.1	32.8	26.15 2
Nicotinamide Adenine Dinucleotide Phosphate (Reduced)	744	159	622.1	53	35.30 8
Para-Amino Benzoic Acid	136. 05	92	NA	15.5	13.47 1
Phenylpyruvate	163. 35	91	101	11.7	39.97 4
Phosphoenolpyruvate	167. 35	78.9	62.9	21.8	25.16 9
Propionyl Coenzyme A	821. 95	408	158.9	34.4	37
Pyruvate	87.2	43	31.65	12.5	13.161
Ribulose 1,5-Bisphosphate	308 .9	97	78.8	21.6	25.01 7
Sedoheptulose 7-Phosphate	289	96.95	78.9	NA	9.269
Shikimate-3-Phosphate	253	96.95	78.95	17.1	23.76 3
Shikimic Acid	173. 05	93.05	111.05	21.6	5.864
Succinate	117. 25	73	98.95	14.3	19.49 9
Succinate D6 (Internal standard)	121	76.9	102.1	14	19.43 8
Succinyl-Coenzyme A	866	426.1	339.2	37.2	38.5
Uridine Diphosphate	403	159.1	111.05	26.2	25.04
Uridine Diphosphate Glucuronic Acid	578. 95	403	323.1	23.6	34.75 4



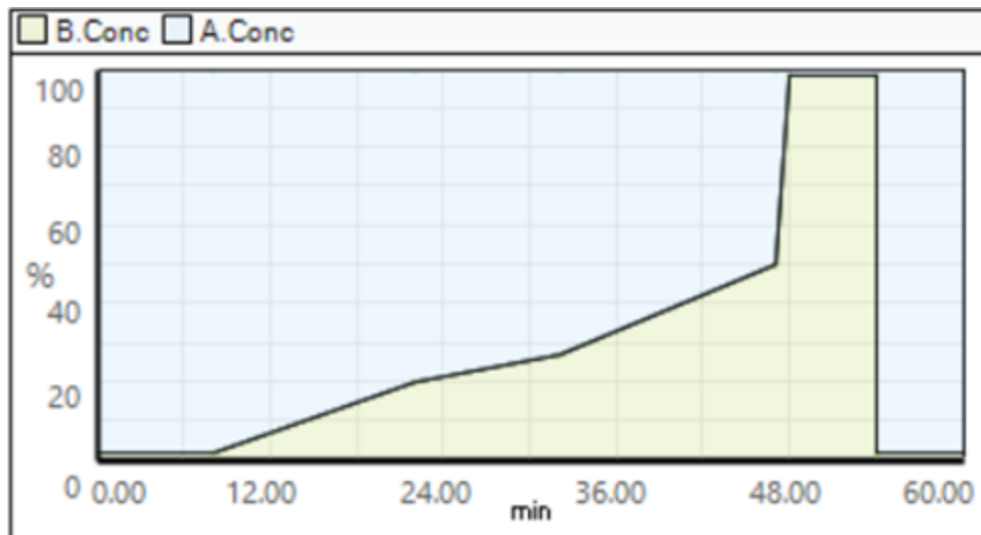
	A	B	C	D	E	F
	Uridine Diphosphate N-Acetylglucosamine	606 .05	385.05	159.1	28	24.43 7
	Uridine Monophosphate	323 .05	96.95	79.05	23	14.32 6
	Uridine Triphosphate	482 .95	159	385.05	27.4	35.44 5

20 MS/MS Settings

- Acquisition settings: negative ionisation mode
- Nebulizing Gas flow: 3 L/min
- Heating gas flow: 10 L/min
- Drying gas flow: 10 L/min
- Interface temperature:  300 °C
- Desolvation line temperature:  250 °C

21 LC gradient protocol

A	B
Time	Mobile phase B (%)
8	2
22	20
32	27
47	50
48	98
54	98
60	2



LC Gradient

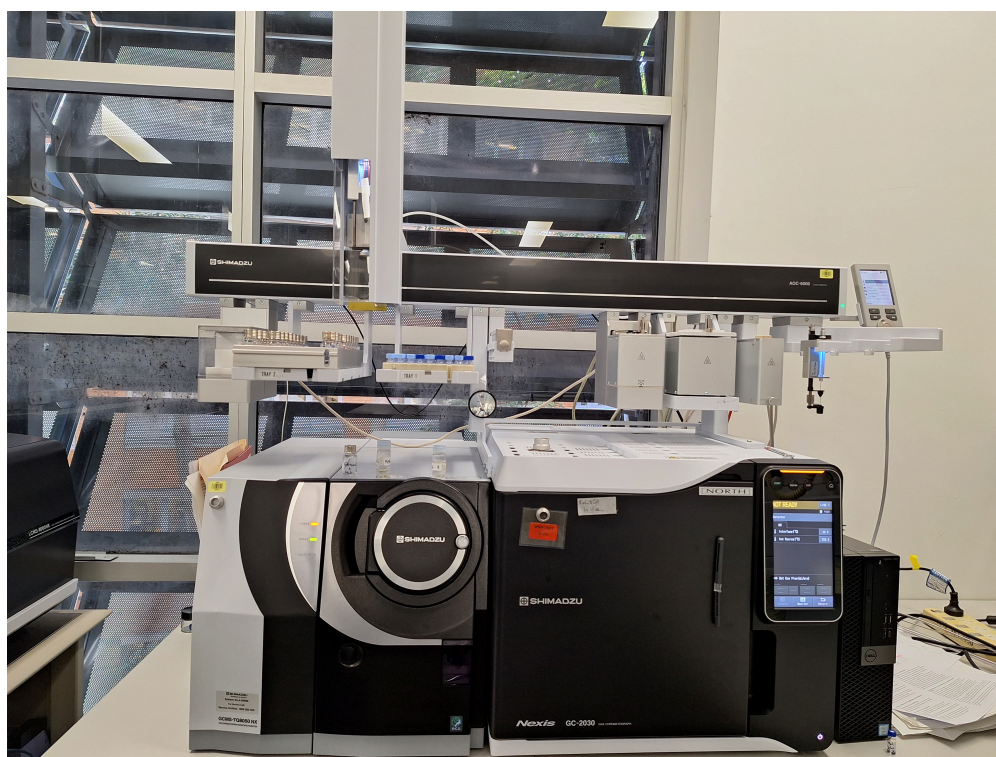
- 22 An injection volume of 5 μ L was utilized for each sample, with a flow rate maintained at 0.3 mL/min throughout the procedure. The mobile phases consisted of Mobile Phase A: 7.5 mM Tritbutylamine adjusted with acetic acid to a pH of 4.95, and Mobile Phase B: 100% acetonitrile. The chromatographic gradient was established as follows: from 0 to 8 minutes, 2% B; 8 to 22 minutes, a gradient increase from 2% to 20% B; 22 to 32 minutes, further increased to 27% B; 32 to 47 minutes, elevated to 50% B; a sharp rise to 98% B at 47 to 48 minutes, held constant at 98% B until 54 minutes, and then reduced back to 2% B from 54 to 60 minutes.

- Total analysis time: 60 min
- Flow: 0.3 mL /min
- Injection volume: 5ul
- Oven temperature: 45 °C

4. GC-MS/MS — Open profiling—amino acids, organic acids, sugars, fatty acids

- 23 To the 500uL samples prepared by DD and AP, add 5uL of 500uM ^{13}C sorbitol. Vortex
- 24 Dry aliquots of this extract, by adding 10 μ L aliquots into a glass insert and drying in a speed vacuum.
- 25 Before analysis, perform a final quick dry down by adding 25 μ L methanol as samples must be completely dry before derivatising with methoxyamine and BSTFA + 1%TMCS.

- 26 Derivatise with methoxyamine (meox) (30mg/ml in pyridine) and BSTFA + 1%TMCS.
- 27 Add 25ul meox solution and incubate at 37 °C for 2 hours. Centrifuge for 10 seconds.
- 28 Add 25uL BSTFA+ 1%TMCS reagent and incubate at 37 °C for 30 minutes. Leave at room temperature for 2 hours before injecting in GCMS system.
- 29 GC-MS analysis was performed on a Shimadzu GC/MS-TQ8050 NX system. 1 µL of derivatised sample was injected into the GC inlet set at 280 °C in split mode of 1:10. Chromatographic separation was achieved using an Agilent DB-5 ms capillary column (30 m × 0.25 mm × 1 µm). Oven conditions were set at 100 °C starting temperature, held for 4 min, then ramped at 10 °C/min to 320 °C and held for 11 min. Helium was used as the carrier gas at a flow rate of 1 mL/min.



Shimadzu GC/MS-TQ8050 NX system



- 30 Compounds were fragmented by electron impact (EI) ionization and analysed in full scan and MRM mode using the Shimadzu Smart Metabolites Database.
(<https://www.shimadzu.com/an/gcms/metabolites/index.html>)
- 31 A high-quality matrix was manually curated using the Shimadzu LabSolutions Insight GCMS program (v.3.7 SP3, Shimadzu Corporation), where metabolite targets were removed from the dataset if they were not present in all samples.

Protocol references

- Valgepea, K., et al. Arginine deiminase pathway provides ATP and boosts growth of the gas-fermenting acetogen *Clostridium autoethanogenum*. *Metab Eng*, (2017). **41**: p. 202-211. <https://doi.org/10.1016/j.ymben.2017.04.007>
- Wood, J.C., et al. Characterisation of acetogen formatotrophic potential using *Eubacterium limosum*. *Appl Microbiol Biotechnol* **107**, 4507–4518 (2023). <https://doi.org/10.1007/s00253-023-12600-6>
- Heffernan, J., et al. Adaptive laboratory evolution of *Clostridium autoethanogenum* to metabolize CO₂ and H₂ enhances growth rates in chemostat and unravels proteome and metabolome alterations. *Microbial Biotechnology*, (2024) **17**, e14452. <https://doi.org/10.1111/1751-7915.14452>
- Forwood, D.L., Innes, D.J., Parra, M.C. *et al.* Feeding an unsalable carrot total-mixed ration altered bacterial amino acid degradation in the rumen of lambs. *Sci Rep* **13**, 6942 (2023). <https://doi.org/10.1038/s41598-023-34181-0>