

Additional file 1

High-throughput quantification of more than 100 primary- and secondary-metabolites, and phytohormones by a single solid-phase extraction based sample preparation with analysis by UHPLC-HESI-MS/MS

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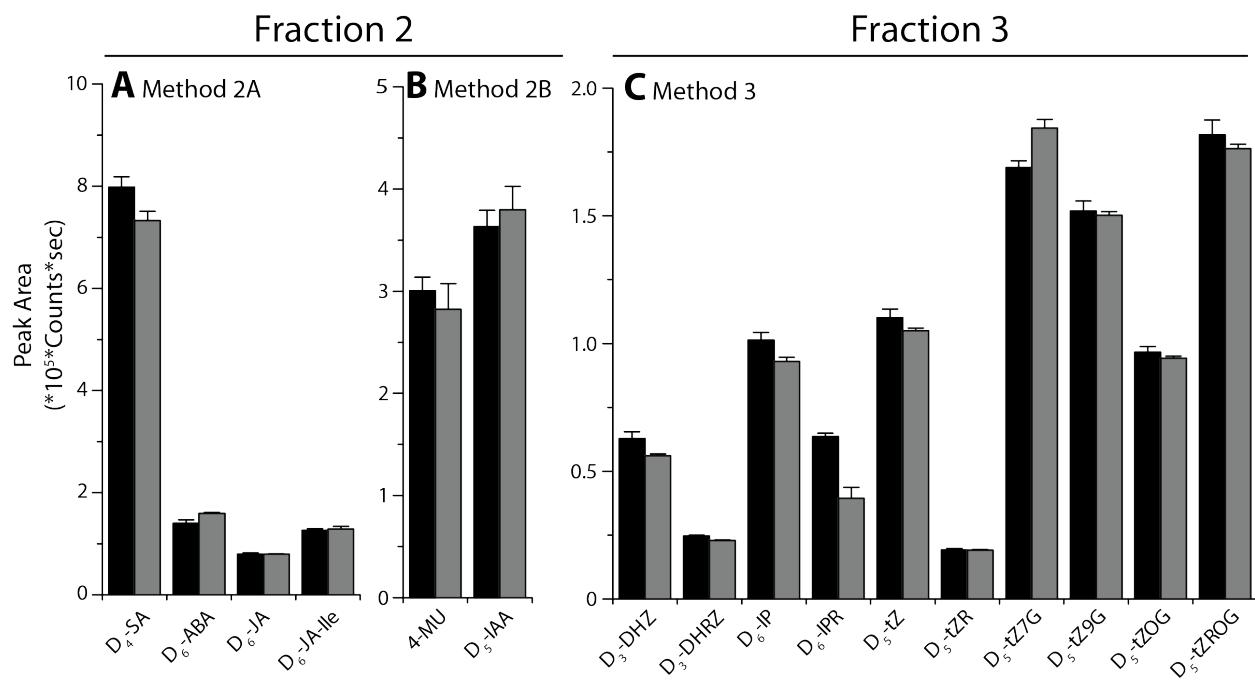


Figure S1 Quality control of multiply used solid phase extraction (SPE) columns.

Average signal intensity (\pm SE; n=4) of isotopically labeled phytohormone standards and 4-methylumbelliferon (4-MU) extracted and purified together with leaf samples two days after simulated herbivory. The purification was done either on a new set of SPE columns (grey bars) or on columns that were used 3 times previously (solid bars). A washing step was included between each use.

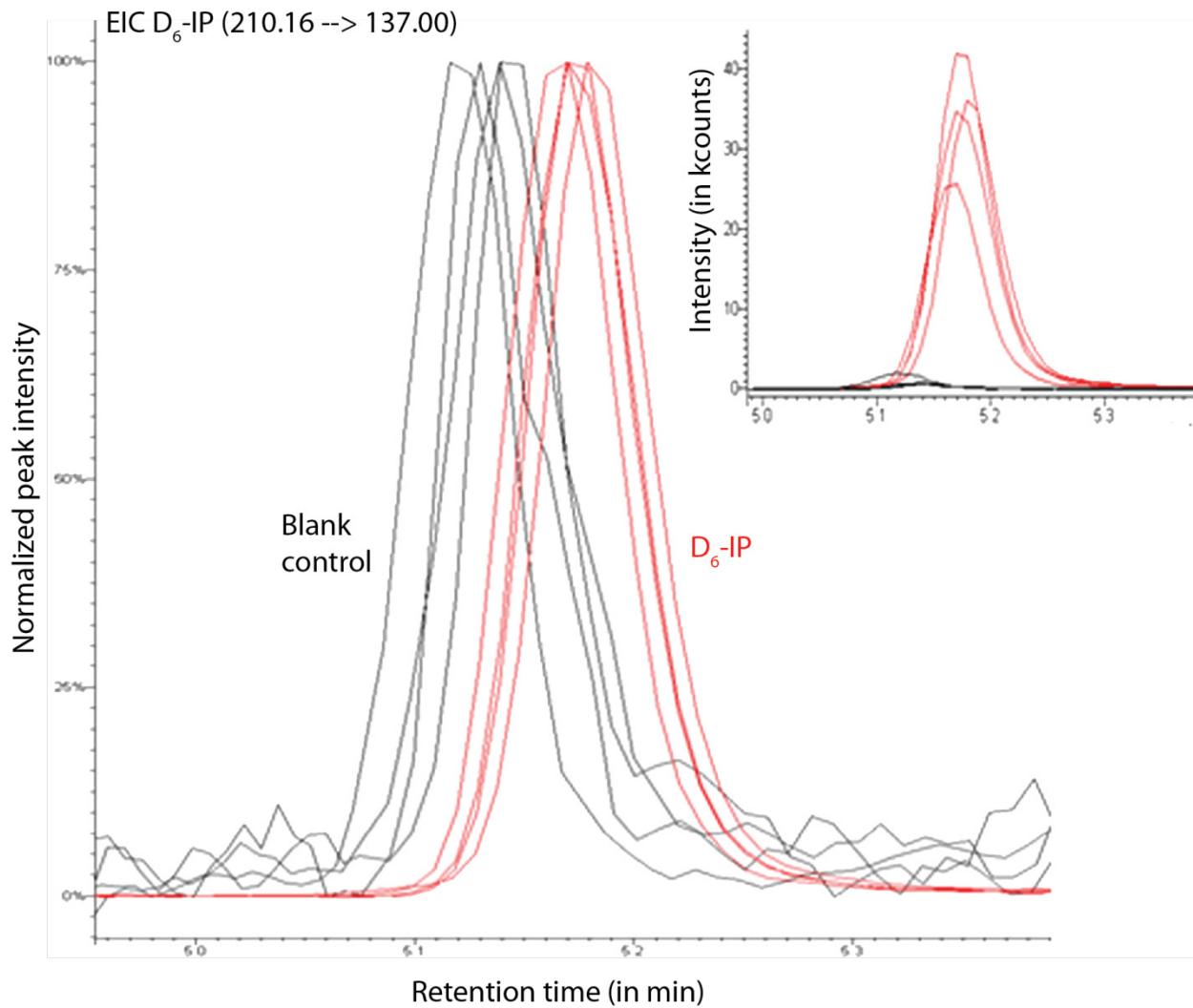


Figure S2 Overlay of the iontrace of the D₆-IP standard and a background noise peak.

D₆-IP trace (+)210.16 → 137.00 from an plant tissue extract and from an equally treated negative control (no tissue and no internal standards added). The figure shows an overlay from each four independent negative controls (black lines) and plant extracts (red lines). In the main chart all samples were normalized to the most intense signal for (+)210.16 → 137.00 within the RT time window (depicted as 100%). The inset shows the absolute signal intensity (kilo counts per second, kCps).

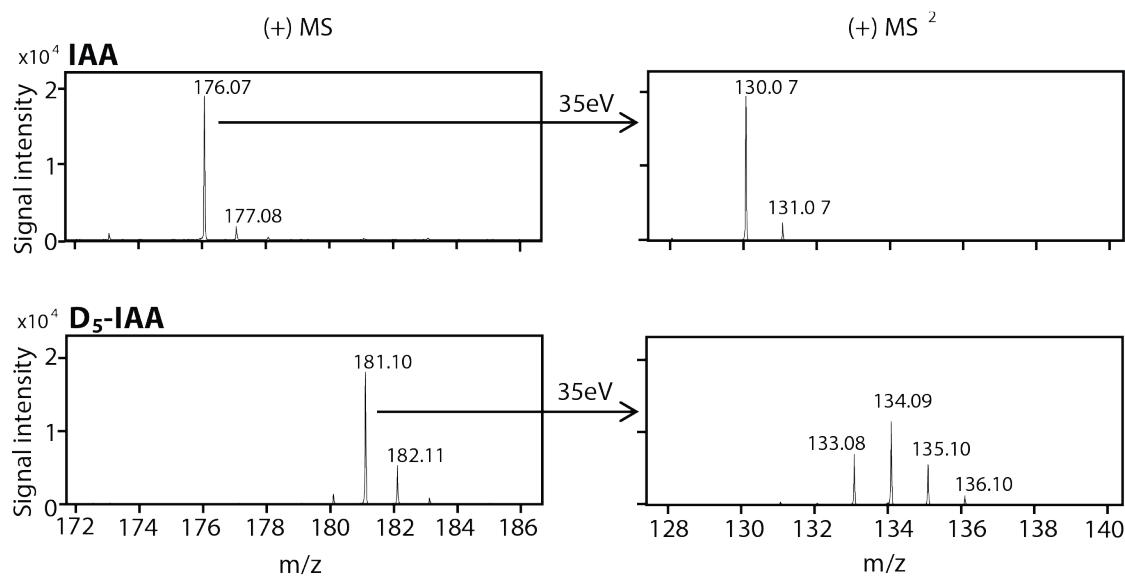


Figure S3 Spectra and fragmentation pattern of isotope labeled and unlabeled IAA.

The MS and MS/MS spectra for IAA and D₅-IAA were acquired on a micrOTOF-Q (Bruker) equipped with an ESI-source. The measurements were performed in the positive ionization mode. For MS/MS analysis the ions 176.07 and 181.10 were fragmented with 35eV.

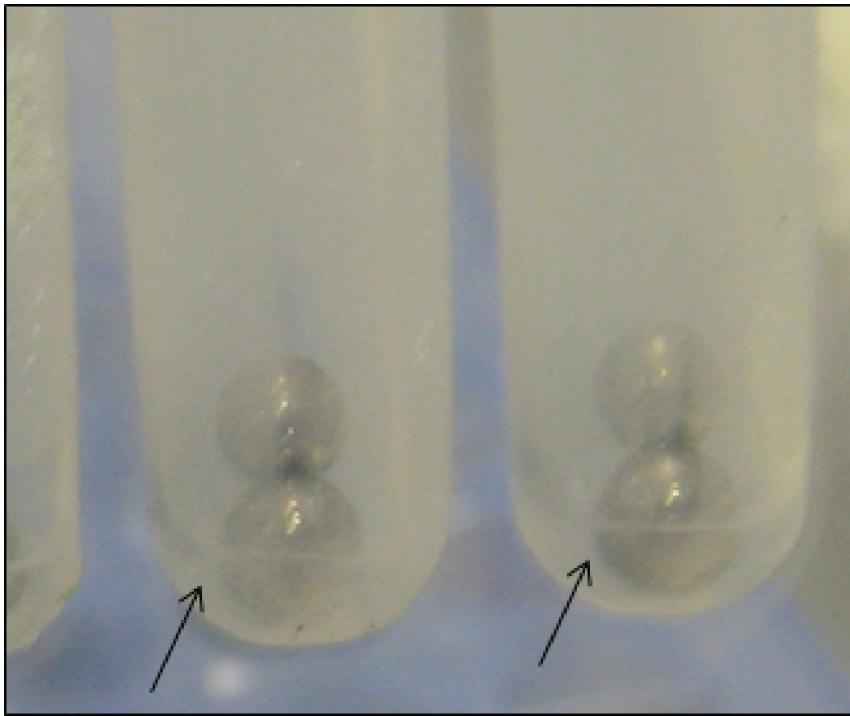


Figure S4 Condensation of air components in 96-well tubes after incubation on N_{2(l)}.

Black arrows indicate the liquid (presumably oxygen) that accumulates at the bottom of the tubes after short incubation on N_{2(l)}. The time until visible liquid accumulation varies depending on how deep the tubes are covered with N_{2(l)}. The tubes were closed with 8-plug caps.

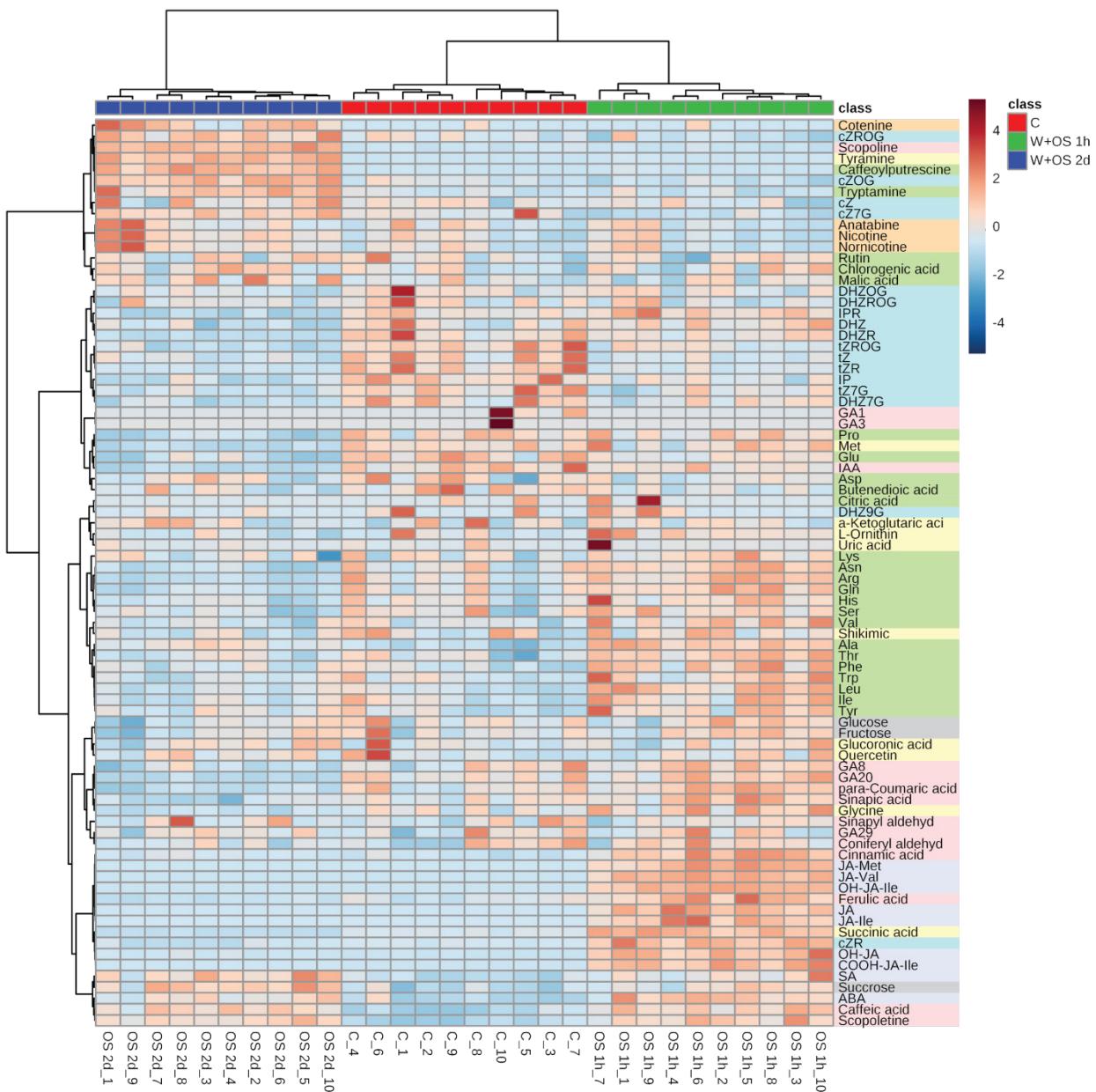


Figure S5 Overview of the metabolite distribution in *N. attenuata* leaves after herbivory.

Heatmap with hierarchical clustering based on Pearson distance measure and Ward clustering algorithm (without data filtering or additional data normalization). The Figure was generated with MetaboAnalyst 3.0 (<http://www.metaboanalyst.ca>, access date: 13.04.2016). Metabolites were analyzed in leaf tissues 1 h and 2 d after artificial herbivory (W+OS 1h and W+OS 2d, respectively), as well as in untreated control samples (C). Individual biological replicates are presented in rows and compounds in

columns. The absolute values of the metabolites are shown in Table S24. Only metabolites that appeared in at least one of the treatment types are shown. The *background color* of compound names indicates the specific MS method they are part of (Method 1A *green*, 1B *yellow*, 1C *orange*, 1D *grey*, 2A *blue*, 2B *pink*, 3 *light blue*).

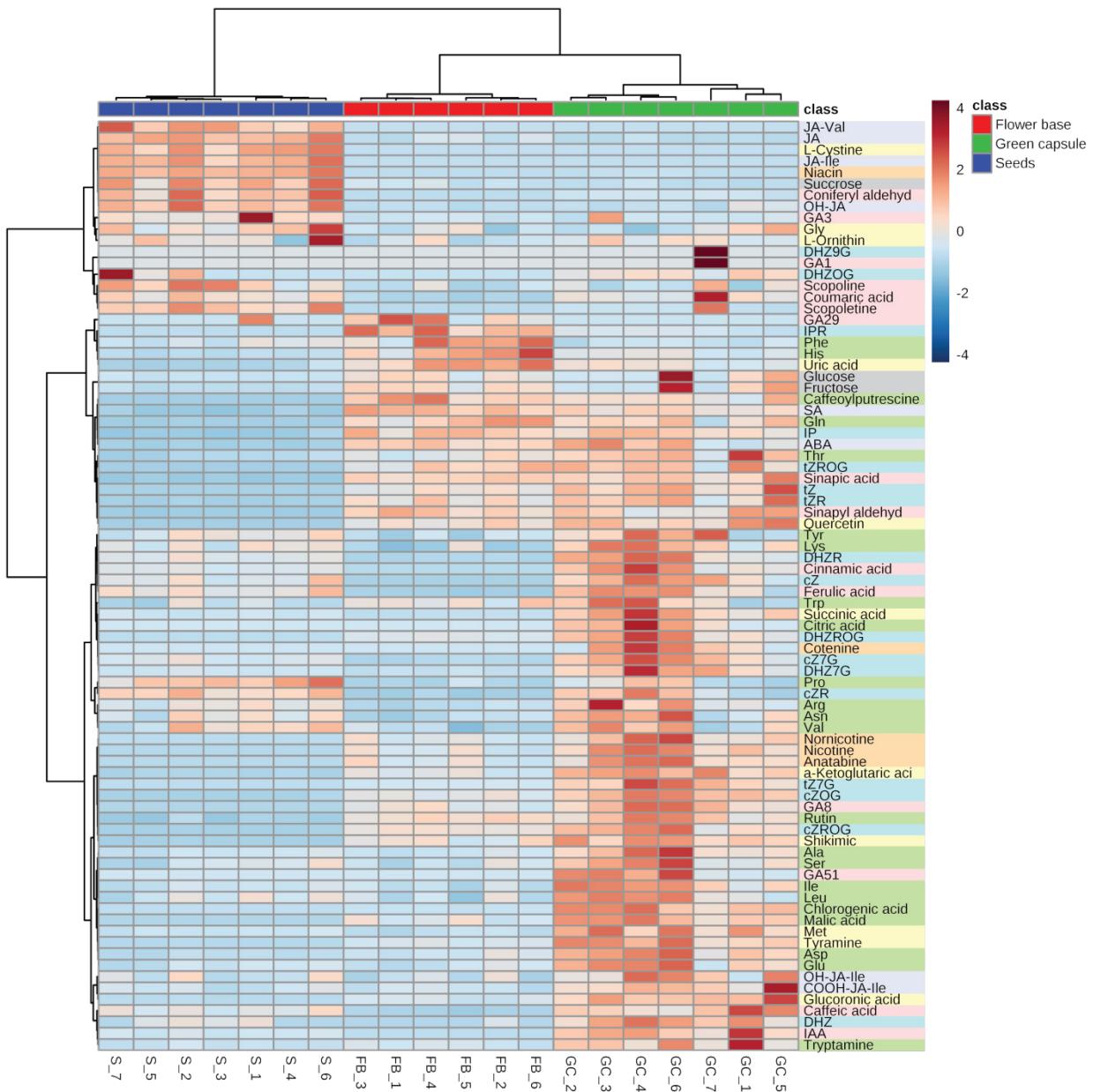


Figure S6 Overview of the metabolite distribution in flower and seed-related tissues of *N. attenuata*.

Heatmap with hierarchical clustering based on Pearson distance measure and Ward clustering algorithm (without data filtering or additional data normalization). The Figure was generated with MetaboAnalyst 3.0 (<http://www.metaboanalyst.ca>, access date: 13.04.2016). Metabolites were analyzed in the lower part of an open flower (Flower base; flower without corolla, stamen, style and pedicel), in developing seed capsules

(Green capsule) and ripe seeds (Seeds). Individual biological replicates are presented in rows and compounds in columns. The absolute values of the metabolites are shown in Table S24. Only metabolites that appeared in at least one of the tissue types are shown. The *background color* of compound names indicates the specific MS method they are part of (Method 1A *green*, 1B *yellow*, 1C *orange*, 1D *grey*, 2A *blue*, 2B *pink*, 3 *light blue*).

Supplemental tables

Table S1 Source parameters

| Method | Spray _{neg} [V] | Spray _{pos} [V] | Cone gas ^a | Probe gas ^a | Nebulizer gas ^b |
|--------|--------------------------|--------------------------|-----------------------|------------------------|----------------------------|
| 1A | 4000 | 4500 | 35/350°C | 60/500°C | 60 |
| 1B | 4000 | 4500 | 35/350°C | 60/500°C | 60 |
| 1C | 0 | 4500 | 25/350°C | 35/300°C | 35 |
| 1D | 4000 | 0 | 35/350°C | 60/500°C | 60 |
| 2A | 4500 | 4500 | 35/350°C | 60/475°C | 60 |
| 2B | 4000 | 4500 | 35/350°C | 60/475°C | 60 |
| 3 | 0 | 4000 | 35/350°C | 60/500°C | 60 |

^a Flow [arbitrary units]/temperature^b Flow [arbitrary units]

Table S2 MRM-settings and retention times of analytes of Method 1A

| Analyte | RT [min] | Q1 [m/z] → | Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|--|-------------|------------|------------------------------------|---------------------|--|
| Ala | 0.62 | (+) | 90.06 → 44.40 | -9 | ¹³ C ₃ , ¹⁵ N ₁ -Ala |
| Arg | 0.63 | (+) | 175.12 → 70.30 | -19 | ¹³ C ₆ , ¹⁵ N ₄ -Arg |
| Asn | 0.63 | (+) | 133.06 → 74.20 | -12 | ¹³ C ₄ , ¹⁵ N _n -Asx _{Asn} ^d |
| Asp | 0.62 | (+) | 134.04 → 74.20 | -12 | ¹³ C ₄ , ¹⁵ N _n -Asx _{Asp} ^d |
| Caffeoylputresine | 2.16 & 2.27 | (+) | 251.14 → 163.00 251.14 → 89.20 | -16 -11 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (0.30) |
| Chlorogenic acid | 2.45 | (+) | 355.10 → 163.00 355.10 → 145.00 | -11 -26 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (0.92) |
| Citric acid | 1.11 | (-) | 191.02 → 111.10 191.02 → 87.10 | 8 13 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (47) |
| Butenedioic acid (fumaric acid & maleic acid) | 1.05 | (-) | 115.00 → 71.20 | 4 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (1.6) |
| Gln | 0.62 | (+) | 147.08 → 130.10 | -6 | ¹³ C ₅ , ¹⁵ N _n -Glx _{Gln} ^d |
| Glu | 0.64 | (+) | 148.06 → 84.20 | -14 | ¹³ C ₅ , ¹⁵ N _n -Glx _{Glu} ^d |
| His | 0.62 | (+) | 156.08 → 110.10 | -11 | ¹³ C ₆ , ¹⁵ N ₃ -His |
| Ile | 1.37 | (+) | 132.10 → 86.20 132.10 → 69.3 | -8 -14 | ¹³ C ₆ , ¹⁵ N ₁ -Ile |
| Leu | 1.46 | (+) | 132.10 → 86.20 | -8 | ¹³ C ₆ , ¹⁵ N ₁ -Leu |
| Lys | 0.60 | (+) | 147.11 → 84.20 | -13 | ¹³ C ₆ , ¹⁵ N ₂ -Lys |
| Malic acid | 0.80 | (-) | 133.01 → 115.10 133.01 → 71.20 | 5 11 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr ^e |
| Phe | 2.18 | (+) | 166.09 → 120.10 | -10 | ¹³ C ₉ , ¹⁵ N ₁ -Phe |
| Pro | 0.69 | (+) | 116.07 → 70.20 | -12 | ¹³ C ₅ , ¹⁵ N ₁ -Pro |
| Rutin | 2.80 | (+) | 611.16 → 302.90 611.16 → 465.00 | -23 -10 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (2.6) |
| Ser | 0.61 | (+) | 106.05 → 60.30 | -8 | ¹³ C ₃ , ¹⁵ N ₁ -Ser |
| Thr | 0.63 | (+) | 120.07 → 74.20 | -8 | ¹³ C ₄ , ¹⁵ N ₁ -Thr |
| Trp | 2.37 | (+) | 205.10 → 188.00 205.10 → 146.00 | -7 -15 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (3.3) |

| | | | | |
|---|------|--------------------|-----|--|
| Tyr | 1.35 | (+)182.08 → 136.10 | -11 | $^{13}\text{C}_9, ^{15}\text{N}_1\text{-Tyr}$ |
| Tyramine | 1.58 | (+)138.10 → 121.10 | -7 | $^{13}\text{C}_9, ^{15}\text{N}_1\text{-Tyr}$ (0.93) |
| | | (+)138.10 → 77.20 | -25 | |
| Val | 0.83 | (+)118.09 → 72.30 | -8 | $^{13}\text{C}_5, ^{15}\text{N}_1\text{-Val}$ |
| $^{13}\text{C}_3, ^{15}\text{N}_1\text{-Ala}$ | 0.60 | (+)94.06 → 47.40 | -9 | |
| $^{13}\text{C}_6, ^{15}\text{N}_4\text{-Arg}$ | 0.62 | (+)185.12 → 75.30 | -19 | |
| $^{13}\text{C}_4, ^{15}\text{N}_n\text{-Asx}$ | 0.61 | (+)139.05 → 77.20 | -12 | |
| $^{13}\text{C}_5, ^{15}\text{N}_n\text{-Glx}$ | 0.62 | (+)154.07 → 89.20 | -14 | |
| $^{13}\text{C}_6, ^{15}\text{N}_3\text{-His}$ | 0.60 | (+)165.09 → 118.10 | -11 | |
| $^{13}\text{C}_6, ^{15}\text{N}_1\text{-Ile}$ | 1.32 | (+)139.12 → 92.20 | -8 | |
| $^{13}\text{C}_6, ^{15}\text{N}_1\text{-Leu}$ | 1.42 | (+)139.12 → 92.20 | -8 | |
| $^{13}\text{C}_6, ^{15}\text{N}_2\text{-Lys}$ | 0.60 | (+)155.12 → 90.20 | -13 | |
| $^{13}\text{C}_9, ^{15}\text{N}_1\text{-Phe}$ | 2.14 | (+)176.11 → 129.10 | -10 | |
| $^{13}\text{C}_5, ^{15}\text{N}_1\text{-Pro}^f$ | 0.67 | (+)122.08 → 75.30 | -12 | |
| | | (+)122.03 → 59.20 | -19 | |
| $^{13}\text{C}_3, ^{15}\text{N}_1\text{-Ser}$ | 0.60 | (+)110.06 → 63.30 | -8 | |
| $^{13}\text{C}_4, ^{15}\text{N}_1\text{-Thr}$ | 0.62 | (+)125.07 → 78.20 | -8 | |
| $^{13}\text{C}_9, ^{15}\text{N}_1\text{-Tyr}$ | 1.34 | (+)192.10 → 145.10 | -11 | |
| $^{13}\text{C}_5, ^{15}\text{N}_1\text{-Val}$ | 0.81 | (+)124.10 → 77.30 | -8 | |

RT: retention time

CE: collision energy

^a Qualifiers are depicted in grey

^b Resolution: Q1: 0.7, Q3: 2

^c Incl. matrix (leaf) and recovery corrected response factor in brackets

^d Asx and Glx handled as Asn, Asp, Glu or Gln equivalents, respectively

^e Relative quantification

^f grey depicted transition is specific for Cys, which could interfere with the $^{13}\text{C}_5, ^{15}\text{N}_1\text{-Pro}$ analysis

Table S3 MRM-settings and retention times of analytes of Method 1B

| Analyte | RT [min] | Q1 [m/z] → | Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|--|----------|-------------|-------------------------|---------------------|---|
| α-Ketoglutaric acid | 0.88 | (-)145.01 → | 57.30 | 8 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (19) |
| | | (-)145.01 → | 101.10 | 4 | |
| Cystine | 0.61 | (+)241.03 | 152.00 | -10 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (5.5) |
| | | (+)241.03 → | 74.20 | -21 | |
| Glucuronic acid | 0.63 | (-)193.04 → | 73.20 | 10 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (34) |
| | | (-)193.04 → | 113.10 | 6 | |
| Gly | 0.61 | (+)76.04 → | 30.50 | -8 | ¹³ C ₂ , ¹⁵ N ₁ -Gly |
| Met | 0.94 | (+)150.06 → | 56.30 | -14 | ¹³ C ₅ , ¹⁵ N ₁ -Met |
| Ornithine | 0.58 | (+)133.10 → | 70.30 | -14 | ¹³ C ₆ , ¹⁵ N ₂ -Tyr (2.4) |
| | | (+)133.10 → | 116.10 | -6 | |
| Quercetin | 3.05 | (-)301.04 → | 151.00 | 18 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (5.8) |
| | | (-)301.04 → | 179.00 | 15 | |
| Shikimic acid | 0.82 | (-)173.05 → | 93.10 | 8 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (112) |
| | | (-)173.05 → | 111.10 | 4 | |
| Succinic acid | 1.41 | (-)117.02 → | 73.20 | 7 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (1.4) |
| | | (-)117.02 → | 99.10 | 5 | |
| Tryptamine | 2.37 | (+)161.11 → | 144.10 | -8 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (0.13) |
| | | (+)161.11 → | 117.10 | -24 | |
| Uracil | 0.93 | (-)111.02 → | 42.40 | 10 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (6.5) |
| | | (-)111.02 → | 80.10 | 17 | |
| Uric acid | 1.10 | (-)167.02 → | 124.10 | 10 | ¹³ C ₉ , ¹⁵ N ₁ -Tyr (86) |
| | | (-)167.02 → | 42.40 | 16 | |
| ¹³ C ₂ , ¹⁵ N ₁ -Gly | 0.60 | (+)79.04 → | 32.50 | -8 | |
| ¹³ C ₅ , ¹⁵ N ₁ -Met | 0.93 | (+)156.07 → | 60.30 | -14 | |
| ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 1.34 | (+)192.10 → | 145.10 | -11 | |
| Tyr ^d | 1.35 | (+)182.08 → | 136.10 | -11 | |

RT: retention time

CE: collision energy

^a Qualifiers are depicted in grey

^b Resolution: Q1: 0.7, Q3: 2

^c Incl. matrix and recovery corrected response factor for leaf material in brackets

^d Usually not included in the method (just for method evaluation of ¹³C₉, ¹⁵N₁-Tyr)

Table S4 MRM-settings and retention times of analytes of Method 1C

| Analyte | RT [min] | Q1 [m/z] → Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|--|----------|------------------------------------|---------------------|--|
| Anabasine | 4.17 | (+)163.12 → 118.10 | -20 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (3.9) |
| | | (+)163.12 → 92.00 | -20 | |
| Anatabine | 3.74 | (+)161.11 → 144.10 | -11 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (0.13) |
| | | (+)161.11 → 80.20 | -22 | |
| | | (+)161.11 → 107.10 | -10 | |
| Cotinine | 2.75 | (+)177.10 → 80.20 | -21 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (0.13) |
| | | (+)177.10 → 98.00 | -20 | |
| Niacin | 0.37 | (+)124.04 → 80.20 | -19 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (5.8) |
| | | (+)124.04 → 78.00 | -18 | |
| Nicotine | 4.27 | (+)163.12 → 130.10 | -17 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (0.38) |
| Nornicotine | 3.69 | (+)149.11 → 80.20 | -18 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (0.083) |
| | | (+)149.11 → 130.10 | -14 | |
| | | (+)149.11 → 117.10 | -22 | |
| Scopolamine | 4.37 | (+)304.15 → 138.10 | -19 | ¹³ C ₉ , ¹⁵ N ₁ -Phe (0.063) |
| | | (+)304.15 → 156.10 | -14 | |
| | | (+)304.15 → 103.10 | -33 | |
| ¹³ C ₉ , ¹⁵ N ₁ -Phe | 0.62 | (+)176.11 → 129.10 | -10 | |
| Phe ^d | 0.62 | (+)166.09 → 120.10 | -10 | |

RT: retention time

CE: collision energy

^a Qualifiers are depicted in grey^b Resolution: Q1: 0.7, Q3: 2^c Incl. matrix (leaf) and recovery corrected response factor in brackets^d Usually not included in the method (just for method evaluation of ¹³C₉, ¹⁵N₁-Phe)

Table S5 MRM-settings and retention times of analytes of Method 1D

| Analyte | RT [min] | Q1 [m/z] → | Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|------------|----------|-------------|-------------------------|---------------------|-----------------------|
| Cellubiose | 7.44 | (-)341.11 → | 161.10 | 3 | Sorbitol (5.8) |
| | | (-)341.11 → | 101.10 | 11 | |
| Fructose | 4.72 | (-)179.06 → | 89.10 | 5 | Sorbitol (0.69) |
| | | (-)179.06 → | 71.20 | 10 | |
| Glucose | 5.71 | (-)179.06 → | 89.10 | 5 | Sorbitol (0.95) |
| | | (-)179.06 → | 71.20 | 7 | |
| Rhamnose | 3.72 | (-)163.06 → | 59.30 | 9 | Sorbitol (1.4) |
| | | (-)163.06 → | 103.10 | 4 | |
| Sucrose | 6.92 | (-)341.11 → | 89.20 | 17 | Sorbitol (2.8) |
| | | (-)341.11 → | 179.00 | 10 | |
| Sorbitol | 5.07 | (-)181.07 → | 59.30 | 16 | |
| | | (-)181.07 → | 71.20 | 14 | |

RT: retention time

CE: collision energy

^a Qualifiers are depicted in grey^b Resolution: Q1: 0.7, Q3: 2^c Incl. matrix (leaf) and recovery corrected response factor in brackets

Table S6 MRM-settings and retention times of analytes of Method 2A

| Analyte | RT [min] | Q1 [m/z] → | Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|------------------------|-------------|-------------|-------------------------|---------------------|-------------------------------------|
| ABA | 2.26 | (-)263.13 → | 153.00 | 9 | D ₄ -ABA |
| COOH-JA-Ile | 2.22 | (-)352.00 → | 130.00 | 19 | D ₆ -JA-Ile ^d |
| JA | 2.52 | (-)209.12 → | 59.00 | 12 | D ₆ -JA |
| JA-Ile | 2.81 | (-)322.20 → | 130.00 | 19 | D ₆ -JA-Ile |
| JA-Met | 2.60 | (-)340.16 → | 148.10 | 13 | D ₆ -JA-Ile (1.83) |
| | | (-)340.16 → | 47.30 | 24 | |
| JA-Phe | 2.79 | (-)356.19 → | 164.10 | 16 | D ₆ -JA-Ile (1.54) |
| | | (-)356.19 → | 147.10 | 26 | |
| JA-Trp | 2.70 | (-)395.20 → | 203.11 | 15 | D ₆ -JA-Ile ^d |
| | | (-)395.20 → | 245.05 | 10 | |
| JA-Val | 2.64 | (-)308.19 → | 116.10 | 16 | D ₆ -JA-Ile (0.49) |
| | | (-)308.19 → | 158.10 | 10 | |
| OH-JA | 1.91 | (-)225.11 → | 59.00 | 12 | D ₆ -JA ^d |
| OH-JA-Ile | 2.25 | (-)338.20 → | 130.00 | 19 | D ₆ -JA-Ile ^d |
| SA | 2.25 | (-)137.02 → | 93.00 | 15 | D ₆ -SA |
| D ₄ -ABA | 2.26 | (-)269.17 → | 159.00 | 10 | |
| D ₆ -JA | 2.51 | (-)215.15 → | 59.00 | 10 | |
| D ₆ -JA-Ile | 2.71 & 2.80 | (-)328.24 → | 130.00 | 19 | |
| D ₆ -SA | 2.24 | (-)141.05 → | 97.00 | 15 | |

RT: retention time

CE: collision energy

^a Qualifiers are depicted in grey^b Resolution: Q1: 0.7, Q3: 2^c Incl. matrix (leaf) and recovery corrected response factor in brackets^d Relative quantification

Table S7 MRM-settings and retention times of analytes of Method 2B

| Analyte | RT [min] | Q1 [m/z] → | Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|-----------------------------|-------------|-------------|-------------------------|---------------------|-----------------------|
| Caffeic acid | 1.77 | (-)179.04 → | 135.10 | 11 | 4-MU (0.47) |
| | | (-)179.04 → | 107.10 | 18 | |
| Cinnamic acid | 4.43 | (-)147.05 → | 103.10 | 7 | 4-MU (4.3) |
| | | (-)147.05 → | 77.20 | 19 | |
| Coniferyl aldehyde | 2.48 | (+)179.07 → | 147.00 | -11 | 4-MU (0.41) |
| | | (+)179.07 → | 91.10 | -24 | |
| <i>para</i> -Coumaric acid | 2.17 & 2.28 | (-)163.04 → | 119.10 | 10 | 4-MU (0.12) |
| | | (-)163.04 → | 93.20 | 26 | |
| <i>meta</i> -Coumaric acid | 2.56 | (-)163.04 → | 119.10 | 10 | 4-MU (0.14) |
| | | (-)163.04 → | 93.20 | 26 | |
| <i>ortho</i> -Coumaric acid | 3.11 | (-)163.04 → | 119.10 | 10 | 4-MU (0.13) |
| | | (-)163.04 → | 93.20 | 26 | |
| Ferulic acid | 2.26 & 2.48 | (-)193.05 → | 134.10 | 11 | 4-MU (0.63) |
| | | (-)193.05 → | 178.00 | 8 | |
| Fraxetin | 1.87 | (-)207.03 → | 191.90 | 11 | 4-MU (0.39) |
| | | (-)207.03 → | 108.10 | 19 | |
| GA ₁ | 2.39 | (-)347.15 → | 259.00 | 17 | 4-MU (1.59) |
| | | (-)347.15 → | 303.00 | 17 | |
| GA ₃ | 2.24 | (-)345.14 → | 143.00 | 26 | 4-MU (1.0) |
| | | (-)345.14 → | 239.00 | 13 | |
| GA ₄ | 6.84 | (-)331.16 → | 243.00 | 13 | 4-MU (0.58) |
| | | (-)331.16 → | 257.00 | 22 | |
| GA ₅ | 4.38 | (-)329.14 → | 145.00 | 19 | 4-MU (2.31) |
| | | (-)329.14 → | 241.10 | 8 | |
| | | (-)329.14 → | 285.00 | 10 | |
| GA ₇ | 6.42 | (-)329.14 → | 223.10 | 14 | 4-MU (0.083) |
| GA ₈ | 1.68 | (-)363.15 → | 275.00 | 13 | 4-MU (16) |
| GA ₉ | 8.24 | (-)315.16 → | 271.00 | 19 | 4-MU (35) |

| | | | | |
|---------------------|-------------|--|----------------|----------------------------|
| GA ₁₂ | 9.74 | (-)331.19 → 313.00 (-)331.19 → 269.00 | 26 32 | 4-MU (88) |
| GA _{12ald} | 9.91 | (-)315.20 → 271.10 (-)315.20 → 163.00 | 21 26 | |
| GA ₁₃ | 4.86 | (-)377.16 → 303.00 (-)377.16 → 359.00 (-)377.16 → 215.10 | 20 11 31 | 4-MU (0.68) |
| GA ₂₀ | 4.63 | (-)331.16 → 287.00 (-)331.16 → 225.00 | 19 20 | 4-MU (5.6) |
| GA ₂₄ | 7.99 | (-)345.17 → 257.00 (-)345.17 → 327.00 | 24 19 | 4-MU (10) |
| GA ₂₉ | 1.77 | (-)347.15 → 259.10 (-)347.15 → 303.00 (-)347.15 → 163.00 | 10 13 19 | 4-MU (71) |
| GA ₄₄ | 5.18 | (-)345.17 → 301.00 (-)345.17 → 273.00 (-)345.17 → 255.00 | 18 21 22 | 4-MU (9.0) |
| GA ₅₁ | 5.67 | (-)331.16 → 243.00 (-)331.16 → 287.00 | 9 15 | 4-MU (0.65) |
| IAA | 2.79 | (+)176.07 → 130.00 | -14 | D ₅ -IAA |
| IA-Ala | 2.37 | (+)247.11 → 130.10 (+)247.11 → 90.20 | -15 -10 | D ₅ -IAA (0.46) |
| IAM | 1.89 | (+)175.09 → 130.10 (+)175.09 → 77.20 | -13 -40 | D ₅ -IAA (0.51) |
| IBA | 4.64 | (+)204.10 → 186.00 (+)204.10 → 130.10 | -9 -23 | D ₅ -IAA (1.5) |
| Scopoletine | 2.13 | (+)193.05 → 178.00 (+)193.05 → 133.00 | -21 -19 | 4-MU (1.2) |
| Scopolin | 1.58 | (+)355.10 → 193.00 | -10 | 4-MU ^d |
| Sinapic acid | 2.25 & 2.49 | (+)225.08 → 207.00 (+)225.08 → 175.00 | -7 -13 | 4-MU (0.47) |

| | | | | |
|--|------|--|-------------------|-------------------|
| Sinapyl aldehyde | 2.44 | (-)207.07 → 192.00 (-)207.07 → 177.00 | 10 17 | 4-MU ^d |
| 4-MU | 2.9 | (+)177.05 → 77.20 (+)177.05 → 121.00 | -32 -21 | |
| D ₅ -IAA ^e | 2.75 | (+)181.10 → 135.00 (+)181.10 → 134.00 (+)181.10 → 133.00 | -14 -14 -14 | |
| D ₂ -GA ₃ ^f | 2.24 | (-)347.14 → 143.00 (-)347.14 → 241.00 | 26 13 | |

RT: retention time

CE: collision energy

4-MU: 4-methylumbelliferon

^a Qualifiers are depicted in grey

^b Resolution: Q1: 0.7, Q3: 2

^c Incl. matrix (leaf) and recovery corrected response factor in brackets

^d Relative quantification

^e Analyzed as the sum of all three transitions

^f Usually not included in the method (just for method evaluation of GA₃)

Table S8 MRM-settings and retention times of analytes of Method 3

| Analyte | RT [min] | Q1 [m/z] → Q3 [m/z] ^{a,b} | CE [V] ^a | Standard ^c |
|---------|-------------|--|---------------------|-----------------------------|
| BAP | 5.36 | (+)226.11 → 91.10 (+)226.11 → 65.20 | -20 -44 | D ₆ -IP (2.0) |
| cZ | 2.53 | (+)220.12 → 136.60 (+)220.12 → 148.30 | -16 -16 | D ₅ -tZ |
| cZR | 4.45 | (+)352.16 → 220.30 (+)352.16 → 136.00 | -16 -25 | D ₅ -tZR |
| cZ7G | 2.16 | (+)382.17 → 220.20 (+)382.17 → 136.00 | -19 -30 | D ₅ -tZ7G |
| cZ9G | 2.66 | (+)382.17 → 220.20 (+)382.17 → 136.00 | -19 -30 | D ₅ -tZ9G |
| cZOG | 2.34 | (+)382.17 → 220.20 (+)382.17 → 136.00 | -19 -30 | D ₅ -tZOG |
| cZROG | 4.02 | (+)514.21 → 382.10 | -17 | D ₅ -tZROG |
| DHZ | 2.36 | (+)222.14 → 136.30 | -16 | D ₃ -DHZ |
| DHZR | 4.16 | (+)354.18 → 222.10 | -16 | D ₃ -DHZR |
| DHZ7G | 2.23 & 2.30 | (+)384.19 → 222.00 | -26 | D ₅ -tZ9G (6.2) |
| DHZ9G | 2.46 | (+)384.19 → 222.00 | -26 | D ₅ -tZ9G (2.3) |
| DHZOG | 2.40 | (+)384.19 → 222.00 | -26 | D ₅ -tZ9G(5.4) |
| DHZROG | 3.98 | (+)516.21 → 222.20 (+)516.21 → 384.00 | -28 -20 | D ₃ -tZROG (1.1) |
| IP | 5.18 | (+)204.12 → 136.00 | -14 | D ₆ -IP |
| IPR | 6.10 | (+)336.17 → 204.30 | -12 | D ₆ -IPR |
| IP9G | 5.17 | (+)366.18 → 204.00 (+)366.18 → 136.00 (+)366.18 → 148.00 | -16 -28 -24 | D ₆ -IP (0.21) |
| tZ | 2.25 | (+)220.12 → 136.60 (+)220.12 → 148.30 | -16 -16 | D ₅ -tZ |
| tZR | 4.04 | (+)352.16 → 220.30 (+)352.16 → 136.00 | -16 -25 | D ₅ -tZR |

| | | | | |
|--------------------------------|------|--|------------|--------------------------------|
| <i>t</i> Z7G | 2.04 | (+)382.17 → 220.20 (+)382.17 → 136.00 | -19 -30 | D ₅ - <i>t</i> Z7G |
| <i>t</i> Z9G | 2.41 | (+)382.17 → 220.20 (+)382.17 → 136.00 | -19 -30 | D ₅ - <i>t</i> Z9G |
| <i>t</i> ZOG | 2.13 | (+)382.17 → 220.20 (+)382.17 → 136.00 | -19 -30 | D ₅ - <i>t</i> ZOG |
| <i>t</i> ZROG | 3.62 | (+)514.21 → 382.10 | -17 | D ₅ - <i>t</i> ZROG |
| D ₃ -DHZ | 2.33 | (+)225.15 → 136.60 | -16 | |
| D ₃ -DHZR | 4.10 | (+)357.19 → 225.30 | -16 | |
| D ₆ -IP | 5.11 | (+)210.16 → 137.00 | -14 | |
| D ₆ -IPR | 6.07 | (+)342.20 → 210.00 | -12 | |
| D ₅ - <i>t</i> Z | 2.22 | (+)225.15 → 136.60 | -16 | |
| D ₅ - <i>t</i> ZR | 3.98 | (+)357.19 → 225.30 | -16 | |
| D ₅ - <i>t</i> Z7G | 2.02 | (+)387.20 → 225.00 | -19 | |
| D ₅ - <i>t</i> Z9G | 2.38 | (+)387.20 → 225.00 | -19 | |
| D ₅ - <i>t</i> ZOG | 2.11 | (+)387.20 → 225.00 | -19 | |
| D ₅ - <i>t</i> ZROG | 3.58 | (+)519.24 → 387.10 | -17 | |

RT: retention time

CE: collision energy

^a Qualifiers are depicted in grey

^b Resolution: Q1: 0.7, Q3: 2

^c Incl. matrix (leaf) and recovery corrected response factor in brackets

Table S9 Solvent settings used for Method 1A

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 99 | 1 | 250 |
| 1.00 | 99 | 1 | 500 |
| 2.70 | 0 | 100 | 500 |
| 3.70 | 0 | 100 | 500 |
| 3.90 | 99 | 1 | 250 |
| 4.90 | 99 | 1 | 250 |

^a 0.1% (v/v) ACN, 0.05% (v/v) HCOOH in water

^b MeOH

Table S10 Solvent settings used for Method 1B

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 99 | 1 | 250 |
| 1.00 | 99 | 1 | 500 |
| 2.70 | 0 | 100 | 500 |
| 3.70 | 0 | 100 | 500 |
| 3.90 | 99 | 1 | 250 |
| 4.90 | 99 | 1 | 250 |

^a 0.1% (v/v) ACN, 0.05% (v/v) HCOOH in water

^b MeOH

Table S11 Solvent settings used for Method 1C

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 90 | 10 | 300 |
| 1.00 | 90 | 10 | 300 |
| 5.00 | 18 | 82 | 300 |
| 5.05 | 0 | 100 | 300 |
| 6.00 | 0 | 100 | 300 |
| 6.05 | 90 | 10 | 400 |
| 7.00 | 90 | 10 | 400 |

^a 0.1% (v/v) NH₄OH (>25% NH₃ in water) in water

^b MeOH

Table S12 Solvent settings used for Method 1D

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 20 | 80 | 1000 |
| 9.50 | 40 | 60 | 1000 |
| 10.00 | 95 | 5 | 1000 |
| 12.00 | 95 | 5 | 1000 |
| 12.50 | 20 | 80 | 1000 |
| 18.00 | 20 | 80 | 1000 |

^a 0.1% (v/v) ACN in water

^b ACN

Table S13 Solvent settings used for Method 2A

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 95 | 5 | 400 |
| 0.50 | 95 | 5 | 400 |
| 0.60 | 50 | 50 | 400 |
| 2.50 | 0 | 100 | 400 |
| 3.50 | 0 | 100 | 400 |
| 3.55 | 95 | 5 | 400 |
| 4.50 | 95 | 5 | 400 |

^a 0.1% (v/v) ACN, 0.05% (v/v) HCOOH in water

^b MeOH

Table S14 Solvent settings used for Method 2B

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 90 | 10 | 500 |
| 0.50 | 90 | 10 | 500 |
| 0.65 | 61 | 39 | 500 |
| 3.00 | 59 | 41 | 500 |
| 3.10 | 50 | 50 | 500 |
| 8.50 | 30 | 70 | 500 |
| 9.00 | 0 | 100 | 500 |
| 10.00 | 0 | 100 | 500 |
| 10.05 | 90 | 10 | 500 |
| 11.00 | 90 | 10 | 500 |

^a 0.1% (v/v) ACN, 0.05% (v/v) HCOOH in water

^b MeOH

Table S15 Solvent settings used for Method 3

| Time [min] | A ^a [%] | B ^b [%] | Flow [μ L/min] |
|------------|--------------------|--------------------|---------------------|
| 0.00 | 95 | 5 | 500 |
| 0.50 | 95 | 5 | 500 |
| 0.70 | 85 | 15 | 500 |
| 3.50 | 75 | 25 | 500 |
| 6.50 | 30 | 70 | 500 |
| 6.70 | 0 | 100 | 500 |
| 7.70 | 0 | 100 | 500 |
| 7.80 | 95 | 5 | 500 |
| 8.80 | 95 | 5 | 500 |

^a 0.1% (v/v) ACN, 0.05% (v/v) HCOOH in water

^b MeOH

Table S16 Validation parameter for Method 1A

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Matrix effect ^c [%] | LOQ _m [nmol] |
|--|---|---|-------------------------------|-----------------------|-----------------------------------|----------------------------|
| Ala | 19 | 68 | ≤ 625 | 1.4 x 10 ⁶ | | 3.7 |
| ¹³ C ₃ , ¹⁵ N ₁ -Ala | | | | | 79 | |
| Arg | 7.6 | 27 | ≤ 2,500 | 2.6 x 10 ⁶ | | 1.8 |
| ¹³ C ₆ , ¹⁵ N ₄ -Arg | | | | | 63 | |
| Asn | 28 | 100 | ≤ 2,500 | 9.0 x 10 ⁵ | | 5.2 |
| ¹³ C ₄ , ¹⁵ N _n -Asx _{Asn} ^d | | | | | 83 | |
| Asp | 8.4 | 30 | ≤ 1,250 | 1.1 x 10 ⁶ | | 1.5 |
| ¹³ C ₄ , ¹⁵ N _n -Asx _{Asp} ^d | | | | | 83 | |
| Caffeoylputresince | 15 | 57 | ≤ 995 | 4.9 x 10 ⁶ | 107 | 2.3 |
| Chlorogenic acid | 18 ^e | 68 ^e | ≤ 706 | 6.1 x 10 ⁶ | 281 | 1.0 |
| Citric acid | 406 ^e | 1330 ^e | ≤ 2,603 | 1.8 x 10 ⁴ | 184 | 31 |
| Butenedioic acid (fumaric acid & maleic acid) | 55 | 194 | ≤ 4,308 ^f | 9.9 x 10 ⁵ | 97 | 8.6 |
| Gln | 58 | 202 | ≤ 3,421 ^f | 1.6 x 10 ⁶ | | 11 |
| ¹³ C ₅ , ¹⁵ N _n -Glx _{Gln} ^d | | | | | 78 | |
| Glu | 2.7 | 9.5 | ≤ 1,250 | 2.4 x 10 ⁶ | | 0.52 |
| ¹³ C ₅ , ¹⁵ N _n -Glx _{Glu} ^d | | | | | 78 | |
| His | 6.0 | 21 | ≤ 2,500 | 3.2 x 10 ⁶ | | 1.7 |
| ¹³ C ₆ , ¹⁵ N ₃ -His | | | | | 54 | |
| Ile | 12 | 45 | ≤ 5,000 ^f | 5.7 x 10 ⁶ | | 0.22 |
| ¹³ C ₆ , ¹⁵ N ₁ -Ile | | | | | 86 | |
| Leu | 11 | 39 | ≤ 5,000 ^f | 6.4 x 10 ⁶ | | 0.22 |
| ¹³ C ₆ , ¹⁵ N ₁ -Leu | | | | | 75 | |
| Lys | 18 | 62 | ≤ 2,500 | 2.9 x 10 ⁶ | | 4.2 |
| ¹³ C ₆ , ¹⁵ N ₂ -Lys | | | | | 64 | |
| Phe | 1.2 | 4.3 | ≤ 5,000 ^f | 1.0 x 10 ⁷ | | 0.17 |
| ¹³ C ₉ , ¹⁵ N ₁ -Phe | | | | | 106 | |
| Pro | 6.9 | 24 | ≤ 5,000 ^f | 5.0 x 10 ⁶ | | 0.99 |

| | | | | | |
|--|-----|--------------------------|-----------------------|-----|------|
| ¹³ C ₅ , ¹⁵ N ₁ -Pro | | | | 104 | |
| Rutin | 2.3 | 7.8 ≤ 819 ^f | 5.4 x 10 ⁵ | 113 | 0.30 |
| Ser | 9.7 | 34 ≤ 2,500 | 1.3 x 10 ⁶ | | 1.9 |
| ¹³ C ₃ , ¹⁵ N ₁ -Ser | | | | 75 | |
| Thr | 2.9 | 10 ≤ 2,500 | 1.3 x 10 ⁶ | | 0.56 |
| ¹³ C ₄ , ¹⁵ N ₁ -Thr | | | | 79 | |
| Trp | 2.1 | 7.9 ≤ 5,000 ^f | 2.7 x 10 ⁶ | 119 | 0.28 |
| Tyr | 0.5 | 2.0 ≤ 5,000 ^f | 1.8 x 10 ⁶ | | 0.10 |
| ¹³ C ₉ , ¹⁵ N ₁ -Tyr | | | | 89 | |
| Tyramine | 53 | 187 ≤ 3,645 ^f | 2.0 x 10 ⁶ | 85 | 9.4 |
| Val | 4.7 | 16 ≤ 5,000 ^f | 4.7 x 10 ⁶ | | 0.59 |
| ¹³ C ₅ , ¹⁵ N ₁ -Val | | | | 118 | |

LOD_i: instrument limit of detection

LOQ_i: instrument limit of quantification

LOQ_m: method limit of quantification (per sample)

^a Compounds that are expected to have the same properties are depicted in grey

^b Amount on column

^c For 1 µL of 50 times diluted Fraction 1

^d Asx and Glx handled as Asn, Asp, Glu or Gln equivalents, respectively

^e Calculated based on only 5 (citric acid) or 6 (chlorogenic acid) dilution steps

^f Not tested until loss of linearity (linear range potentially larger)

Table S17 Validation parameter for Method 1B

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Matrix effect ^c [%] | LOQ _m [nmol] |
|--|---|---|-------------------------------|-----------------------|-----------------------------------|----------------------------|
| α-Ketoglutaric acid | 71 ^d | 256 ^d | ≤ 1711 | 1.1 × 10 ⁵ | 71 | 1.5 |
| Cystine | 3.4 | 12 | ≤ 2500 ^e | 7.7 × 10 ⁵ | 33 | 0.15 |
| Glucuronic acid | 63 | 223 | ≤ 1288 | 5.4 × 10 ⁴ | 75 | 1.3 |
| Gly | 566 ^d | 1884 ^d | ≤ 5000 ^e | 6.7 × 10 ⁴ | | 20 |
| ¹³ C ₂ , ¹⁵ N ₁ -Gly | | | | | 41 | |
| Met | 6.3 | 22 | ≤ 5000 ^e | 1.7 × 10 ⁶ | | 0.073 |
| ¹³ C ₅ , ¹⁵ N ₁ -Met | | | | | 129 | |
| Ornithine | 9.5 | 34 | ≤ 3783 ^e | 1.6 × 10 ⁶ | 36 | 0.40 |
| Quercetin | 18 ^d | 66 ^d | ≤ 1478 ^e | 2.3 × 10 ⁵ | 105 | 0.27 |
| Shikimic acid | 37 | 133 | ≤ 2871 ^e | 6.2 × 10 ⁴ | 20 | 2.8 |
| Succinic acid | 52 | 183 | ≤ 4234 ^e | 1.0 × 10 ⁶ | 94 | 0.83 |
| Tryptamine | 1.8 ^d | 6.3 ^d | ≤ 3121 ^e | 1.0 × 10 ⁷ | 108 | 0.25 |
| Uracil | 6.4 | 23 | ≤ 4461 ^e | 3.1 × 10 ⁵ | 68 | 0.14 |
| Uric acid | 100 ^d | 363 ^d | ≤ 2974 ^e | 2.0 × 10 ⁴ | 83 | 1.9 |
| Tyr ^f | | | | 1.8 × 10 ⁶ | | |
| ¹³ C ₉ , ¹⁵ N ₁ -Tyr | | | | | 79 | |

LOD_i: instrument limit of detectionLOQ_i: instrument limit of quantificationLOQ_m: method limit of quantification (per sample)^a Compounds that are expected to have the same properties are depicted in grey^b Amount on column^c For 10 µL of 50 times diluted Fraction 1^d Calculated based on only 5 (Gly, α-ketoglutaric acid and quercetin) or 6 (tryptamine and uric acid) dilution steps^e Not tested until loss of linearity (linear range potentially larger)^f Usually not included in the method (just for method evaluation of ¹³C₉, ¹⁵N₁-Phe)

Table S18 Validation parameter for Method 1C

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Matrix effect ^c [%] | LOQ _m [pmol] |
|--|---|---|-------------------------------|-----------------------|--------------------------------|----------------------------|
| Anabasine | 25 | 91 | ≤ 3082 ^d | 1.3 × 10 ⁶ | 131 | 2969 |
| Anatabine | 0.79 | 2.9 | ≤ 3121 ^d | 1.2 × 10 ⁷ | 425 | 29 |
| Cotinine | 0.10 | 0.39 | ≤ 709 ^d | 4.7 × 10 ⁷ | 107 | 16 |
| Niacin | 0.37 | 1.4 | ≤ 4061 ^d | 1.6 × 10 ⁶ | 73 | 81 |
| D ₃ -Nicotine ^e | 17 | 58 | ≤ 3026 ^d | 4.3 × 10 ⁶ | 418 | 593 |
| Nicotine | | | | | | |
| Nornicotine | 3.6 ^f | 13 ^f | ≤ 3374 ^d | 1.3 × 10 ⁷ | 623 | 90 |
| Scopolamine | 0.046 | 0.17 | ≤ 1141 ^d | 4.2 × 10 ⁷ | 252 | 2.9 |
| Phe ^e | | | | 6.9 × 10 ⁶ | | |
| ¹³ C ₉ , ¹⁵ N ₁ -Phe | | | | | 97 | |

LOD_i: instrument limit of detectionLOQ_i: instrument limit of quantificationLOQ_m: method limit of quantification (per sample)^a Compounds that are expected to have the same properties are depicted in grey^b Amount on column^c For 1 µL of 50 times diluted Fraction 1^d Not tested until loss of linearity (linear range potentially larger)^e Usually not included in the method (just for method evaluation of nicotine and ¹³C₉, ¹⁵N₁-Phe, respectively)^f Calculated based on only 6 dilution steps

Table S19 Validation parameter for Method 1D

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Matrix effect ^c [%] | LOQ _m [nmol] |
|----------------------|---|---|-------------------------------|-------------------|-----------------------------------|----------------------------|
| Cellubiose | 41 | 149 | $\leq 2921^d$ | 1.4×10^5 | 103 | 62 |
| Fructose | 97 | 347 | $\leq 5551^d$ | 1.2×10^6 | 99 | 149 |
| Glucose | 87 | 313 | $\leq 5551^d$ | 8.8×10^5 | 99 | 135 |
| Rhamnose | 95 | 342 | $\leq 6092^d$ | 6.0×10^5 | 96 | 152 |
| Sucrose | 67 | 245 | $\leq 2921^d$ | 2.7×10^5 | 108 | 97 |
| Sorbitol | 71 | 260 | $\leq 5488^d$ | 8.6×10^5 | 96 | 115 |

LOD_i: instrument limit of detectionLOQ_i: instrument limit of quantificationLOQ_m: method limit of quantification (per sample)^a Compounds that are expected to have the same properties are depicted in grey^b Amount on column^c For 1 µL of 500 times diluted Fraction 1^d Not tested until loss of linearity (linear range potentially larger)

Table S20 Validation parameter for Method 2A

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Recovery rate ^c [%] | Matrix effect ^d [%] | LOQ _m [fmol] |
|------------------------|---|---|-------------------------------|-------------------|-----------------------------------|-----------------------------------|----------------------------|
| JA-Met | 0.20 | 0.74 | ≤ 732 | 3.8×10^6 | 51 | 133 | 1173 |
| JA-Phe | 0.12 | 0.44 | ≤ 699 | 7.8×10^6 | 39 | 100 | 1223 |
| JA-Val | 0.18 | 0.65 | ≤ 808 | 1.1×10^7 | 72 | 119 | 826 |
| D ₄ -ABA | 0.21 | 0.78 | ≤ 924 | 5.4×10^6 | 98 | 94 | 922 |
| ABA | | | | | | | |
| D ₆ -JA | 0.17 | 0.61 | ≤ 1156 | 3.5×10^6 | 83 | 97 | 828 |
| JA | | | | | | | |
| D ₆ -JA-Ile | 0.18 | 0.67 | ≤ 759 | 8.2×10^6 | 62 | 92 | 1275 |
| JA-Ile | | | | | | | |
| D ₆ -SA | 0.20 | 0.75 | ≤ 1759 | 2.3×10^7 | 86 | 111 | 848 |
| SA | | | | | | | |

LOD_i: instrument limit of detectionLOQ_i: instrument limit of quantificationLOQ_m: method limit of quantification (per sample)^a Compounds that are expected to have the same properties are depicted in grey^b Amount on column^c Recovery rate for the purification procedure, including all solid phase extraction and evaporation steps^d For 1 µL of Fraction 2

Table S21 Validation parameter for Method 2B

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Recovery rate ^c [%] | Matrix effect ^d [%] | LOQ _m [fmol] |
|------------------------------|---|---|-------------------------------|-----------------------|-----------------------------------|-----------------------------------|----------------------------|
| Caffeic acid | 28 | 102 | ≤ 2775 | 1.6 × 10 ⁶ | 90 | 20 | 6501 |
| Cinnamic acid | 247 ^e | 850 ^e | ≤ 3375 ^f | 8.6 × 10 ⁴ | 38 | 98 | 26080 |
| Coniferyl aldehyde | 8.2 | 31 | ≤ 2806 ^f | 7.3 × 10 ⁵ | 63 | 69 | 764 |
| <i>para</i> -Coumaric acid | 3.7 | 14 | ≤ 3046 ^f | 2.6 × 10 ⁶ | 88 | 50 | 355 |
| <i>meta</i> -Coumaric acid | 5.5 | 20 | ≤ 3046 ^f | 1.7 × 10 ⁶ | 98 | 56 | 424 |
| <i>ortho</i> -Coumaric acid | 9.1 | 33 | ≤ 3046 ^f | 1.8 × 10 ⁶ | 110 | 54 | 635 |
| Ferulic acid | 13 | 48 | ≤ 2575 ^f | 3.9 × 10 ⁵ | 114 | 49 | 979 |
| Fraxetin | 5.8 | 21 | ≤ 2402 ^f | 7.8 × 10 ⁵ | 92 | 50 | 516 |
| GA ₁ | 10 | 36 | ≤ 1435 ^f | 2.0 × 10 ⁵ | 90 | 49 | 939 |
| GA ₄ | 2.1 | 6.6 | ≤ 1053 ^f | 4.4 × 10 ⁵ | 69 | 78 | 140 |
| GA ₅ | 39 ^e | 138 ^e | ≤ 757 ^f | 6.1 × 10 ⁴ | 89 | 111 | 1602 |
| GA ₇ | 0.47 | 1.7 | ≤ 545 ^f | 3.1 × 10 ⁶ | 76 | 70 | 36 |
| GA ₈ | 17 ^e | 63 ^e | ≤ 686 ^f | 4.2 × 10 ⁴ | 63 | 32 | 3584 |
| GA ₉ | 22 ^e | 81 ^e | ≤ 790 ^f | 3.6 × 10 ⁴ | 14 | 79 | 8421 |
| GA ₁₂ | 9.4 | 34 | ≤ 752 ^f | 5.6 × 10 ⁴ | 14 | 20 | 13922 |
| GA _{12ald} | | | | 0.64 | | | |
| GA ₁₃ | 29 | 104 | ≤ 661 ^f | 1.8 × 10 ⁵ | 92 | 126 | 1021 |
| GA ₂₀ | 24 ^e | 86 ^e | ≤ 752 ^f | 2.7 × 10 ⁴ | 87 | 106 | 1060 |
| GA ₂₄ | 40 ^e | 141 ^e | ≤ 722 ^f | 2.9 × 10 ⁴ | 49 | 99 | 3322 |
| GA ₂₉ | 59 ^e | 199 ^e | ≤ 718 ^f | 2.1 × 10 ⁴ | 77 | 12 | 24651 |
| GA ₄₄ | 29 ^e | 104 ^e | ≤ 722 ^f | 1.6 × 10 ⁴ | 83 | 113 | 1267 |
| GA ₅₁ | 11 | 40 | ≤ 752 ^f | 2.4 × 10 ⁵ | 82 | 109 | 514 |
| IA-Ala | 3.1 | 11 | ≤ 2030 ^f | 1.9 × 10 ⁶ | 107 | 84 | 143 |
| IAM | 0.90 | 3.3 | ≤ 1435 | 2.7 × 10 ⁶ | 82 | 69 | 67 |
| IBA | 2.7 | 10 | ≤ 2460 ^f | 1.4 × 10 ⁶ | 49 | 78 | 302 |
| Scopoletine | 7.9 | 29 | ≤ 2602 ^f | 2.2 × 10 ⁵ | 77 | 69 | 631 |
| Sinapic acid | 101 ^e | 358 ^e | ≤ 2230 ^f | 6.8 × 10 ⁵ | 102 | 42 | 9641 |
| Sinapyl aldehyd ^g | | | | | 68 | 13 | |

| | | | | | | | |
|--|-----|-----|---------------|-------------------|----|----|------|
| 4-MU | 36 | 131 | $\leq 2838^f$ | 2.2×10^5 | 96 | 65 | 2393 |
| D ₅ -IAA | 13 | 49 | $\leq 499^f$ | 6.0×10^5 | 88 | 26 | 2425 |
| IAA | 3.8 | 14 | | | | | 679 |
| D ₂ -GA ₃ ^h | 5.0 | 19 | $\leq 1435^f$ | 3.8×10^5 | 83 | 44 | 583 |
| <u>GA₃</u> | | | | | | | |

LOD_i: instrument limit of detection

LOQ_i: instrument limit of quantification

LOQ_m: method limit of quantification (per sample)

4-MU: 4-methylumbelliferon

^a Compounds that are expected to have the same properties are depicted in grey

^b Amount on column

^c Recovery rate for the purification procedure, including all solid phase extraction and evaporation steps, excluding the removal of an aliquot for the analysis by Method 2A

^d For 5 µL of 20 times concentrated Fraction 2

^e Calculated based on only 5 (GA₂₄ and GA₂₉) or 6 (cinnamic acid, sinapic acid, GA₅, GA₈, GA₉, GA₂₀ and GA₄₄) dilution steps

^f Not tested until loss of linearity (linear range potentially larger)

^g Tested with standard of unknown concentration

^h Usually not included in the method (just for method evaluation of GA₃)

Table S22 Validation parameter for Method 3

| Analyte ^a | LOD _i ^b [fmol] | LOQ _i ^b [fmol] | Linear ^b [fmol] | Slope | Recovery rate ^c [%] | Matrix effect ^d [%] | LOQ _m [fmol] |
|----------------------|---|---|-------------------------------|-----------------------|-----------------------------------|-----------------------------------|----------------------------|
| BAP | 2.1 | 7.2 | ≤ 1110 | 8.3 × 10 ⁶ | 4 | 77 | 4621 |
| DHZ7G | 0.38 | 1.4 | ≤ 1304 ^e | 1.2 × 10 ⁶ | 80 | 55 | 58 |
| DHZ9G | 0.35 | 1.3 | ≤ 1304 ^e | 2.7 × 10 ⁶ | 88 | 60 | 45 |
| DHZOG | 0.27 | 0.99 | ≤ 1304 ^e | 1.4 × 10 ⁶ | 67 | 66 | 43 |
| DHZROG | 0.39 | 1.4 | ≤ 970 ^e | 2.0 × 10 ⁶ | 84 | 63 | 49 |
| IP9G | 0.41 | 1.5 | ≤ 684 | 4.7 × 10 ⁶ | 66 | 77 | 55 |
| D ₃ -DHZ | 0.15 | 0.55 | ≤ 1115 ^e | 9.1 × 10 ⁶ | 33 | 40 | 79 |
| DHZ | | | | | | | |
| D ₃ -DHZR | 0.11 | 0.39 | ≤ 140 ^e | 2.1 × 10 ⁷ | 70 | 42 | 25 |
| DHZR | | | | | | | |
| D ₆ -IP | 0.076 | 0.27 | ≤ 478 ^e | 2.6 × 10 ⁷ | 3 | 60 | 264 |
| IP | | | | | | | |
| D ₆ -IPR | 0.032 | 0.11 | ≤ 293 ^e | 2.7 × 10 ⁷ | 15 | 76 | 19 |
| IPR | | | | | | | |
| D ₅ -tZ | 0.18 | 0.66 | ≤ 2230 ^e | 6.7 × 10 ⁶ | 33 | 50 | 78 |
| tZ | | | | | | | |
| cZ | | | | | | | |
| D ₅ -tZR | 0.21 | 0.74 | ≤ 281 ^e | 7.0 × 10 ⁶ | 75 | 59 | 32 |
| tZR | | | | | | | |
| cZR | | | | | | | |
| D ₅ -tZ7G | 0.12 | 0.41 | ≤ 2588 ^e | 7.8 × 10 ⁶ | 85 | 46 | 20 |
| tZ7G | | | | | | | |
| cZ7G | | | | | | | |
| D ₅ -tZ9G | 0.20 | 0.72 | ≤ 2588 ^e | 5.7 × 10 ⁶ | 92 | 64 | 23 |
| tZ9G | | | | | | | |
| cZ9G | | | | | | | |
| D ₅ -tZOG | 0.25 | 0.87 | ≤ 5176 ^e | 2.2 × 10 ⁶ | 71 | 57 | 41 |

| | | | | | | | |
|--------------------------------|------|-----|---------------------|-----------------------|----|----|----|
| <i>t</i> ZOG | | | | | | | |
| <i>c</i> ZOG | | | | | | | |
| D ₅ - <i>t</i> ZROG | 0.42 | 1.5 | ≤ 7714 ^e | 2.2 × 10 ⁶ | 85 | 62 | 54 |
| <i>t</i> ZROG | | | | | | | |
| <i>c</i> ZROG | | | | | | | |

LOD_i: instrument limit of detection

LOQ_i: instrument limit of quantification

LOQ_m: method limit of quantification (per sample)

^a Compounds that are expected to have the same properties are depicted in grey

^b Amount on column

^c Recovery rate for the purification procedure, including all solid phase extraction and evaporation steps

^d For 3 µL of 20 times concentrated Fraction 3

^e Not tested until loss of linearity (linear range potentially larger)

Table S23 Re-analysis of selected samples^a after a prolonged storage time^b

| | Compound abundance relative to first analysis [%] | SE | | Compound abundance relative to first analysis [%] | SE |
|--------------------|---|-----|---------------------|---|-----|
| Method 1A | | | | | |
| Ala | 103 | 5.6 | Gly | 109 | 1.9 |
| Arg | 110 | 13 | Met | 101 | 1.2 |
| Asn | 106 | 2.2 | L-Ornithin | 137 | 26 |
| Asp | 94 | 2.2 | Tryptamine | 191 | 32 |
| Glu | 108 | 5.3 | α-Ketoglutaric acid | 71 | 4 |
| Gln | 126 | 9.1 | Glucoronic acid | 96 | 17 |
| His | 88 | 8.7 | Succinic acid | 116 | 3.0 |
| Ile | 112 | 15 | Uric acid | 79 | 6.9 |
| Leu | 130 | 29 | Quercetin | 174 | 81 |
| Lys | 160 | 33 | Shikimic acid | 223 | 43 |
| Phe | 106 | 8.3 | | | |
| Pro | 108 | 9.1 | | | |
| Ser | 105 | 3.4 | Method 1B | | |
| Thr | 102 | 2.6 | Nicotine | 101 | 3.4 |
| Trp | 80 | 7.0 | Nornicotine | 117 | 2.1 |
| Tyramine | 196 | 11 | Anatabine | 122 | 4.3 |
| Tyr | 114 | 11 | | | |
| Val | 106 | 7.6 | | | |
| Caffeoylputrescine | 162 | 18 | Method 1C | | |
| Chlorogenic acid | 171 | 35 | Nicotine | 106 | 14 |
| Rutin | 166 | 18 | Nornicotine | 100 | 7.4 |
| Butenedioic acid | 98 | 10 | Anatabine | 112 | 13 |
| Malic acid | 98 | 17 | | | |

SE: Standard error

^a Five biological replicates of leave samples after 1 hour simulated herbivory^b Between the analyzes samples were kept for > 1 d at 10°C, > 20 weeks at -20°C and faced additional melting-freezing cycles

Table S24 Data from two example experiments examining metabolic responses of leaves to herbivory and during seed development

| Analyte | Method | Unit | Experiment 1 (herbivory response) | | | | | | Experiment 2 (seed development) | | | | | |
|------------|--------|---------------------------|-----------------------------------|--------|---------|-------|---------|-------|---------------------------------|------|---------------|------|---------|-------|
| | | | C | | W+OS 1h | | W+OS 2d | | Flower base ^a | | Green capsule | | Seeds | |
| | | | Average | SE | Average | SE | Average | SE | Average | SE | Average | SE | Average | SE |
| Ala | 1A | nmol * g FW ⁻¹ | 269 | 19 | 566 | 22 | 433 | 17 | 568 | 52 | 398 | 61 | 220 | 15 |
| Arg | 1A | nmol * g FW ⁻¹ | 64 | 8.9 | 82 | 5.7 | 29 | 1.4 | 117 | 15 | 114 | 18 | 154 | 7.0 |
| Asn | 1A | nmol * g FW ⁻¹ | 206 | 21 | 238 | 10 | 118 | 6.7 | 1606 | 179 | 917 | 141 | 1512 | 137 |
| Asp | 1A | nmol * g FW ⁻¹ | 1232 | 56 | 970 | 34 | 1057 | 22 | 2970 | 265 | 1572 | 119 | 529 | 24 |
| Gln | 1A | nmol * g FW ⁻¹ | 3737 | 488 | 4504 | 293 | 2278 | 136 | 51278 | 4252 | 5995 | 1056 | 653 | 54 |
| Glu | 1A | nmol * g FW ⁻¹ | 3627 | 153 | 2542 | 67 | 1980 | 125 | 6681 | 362 | 2625 | 190 | 2419 | 82 |
| His | 1A | nmol * g FW ⁻¹ | 42 | 3.0 | 48 | 4.6 | 32 | 0.76 | 2324 | 338 | 176 | 10 | 135 | 11 |
| Ile | 1A | nmol * g FW ⁻¹ | 28 | 2.0 | 39 | 2.3 | 25 | 1.2 | 197 | 34 | 123 | 11 | 104 | 3.7 |
| Leu | 1A | nmol * g FW ⁻¹ | 56 | 3.2 | 98 | 4.2 | 55 | 2.3 | 478 | 69 | 171 | 7.4 | 263 | 14 |
| Lys | 1A | nmol * g FW ⁻¹ | 105 | 8.2 | 95 | 3.7 | 88 | 6.8 | 231 | 40 | 141 | 11 | 205 | 15 |
| Phe | 1A | nmol * g FW ⁻¹ | 74 | 5.6 | 121 | 4.4 | 85 | 1.8 | 1070 | 204 | 56 | 6.4 | 203 | 7.2 |
| Pro | 1A | nmol * g FW ⁻¹ | 75 | 7.0 | 75 | 3.9 | 48 | 2.4 | 835 | 140 | 383 | 88 | 2579 | 78 |
| Ser | 1A | nmol * g FW ⁻¹ | 709 | 52 | 728 | 43 | 539 | 21 | 2094 | 194 | 807 | 88 | 888 | 50 |
| Thr | 1A | nmol * g FW ⁻¹ | 349 | 23 | 428 | 14 | 321 | 10 | 2288 | 156 | 642 | 127 | 292 | 9.1 |
| Trp | 1A | nmol * g FW ⁻¹ | 12 | 1.8 | 21 | 2.4 | 12 | 1.1 | 2672 | 350 | 413 | 58 | 806 | 95 |
| Tyr | 1A | nmol * g FW ⁻¹ | 14 | 1.2 | 22 | 1.6 | 15 | 0.76 | 134 | 20 | 57 | 14 | 103 | 3.4 |
| Tyramine | 1A | nmol * g FW ⁻¹ | 17 | 1.4 | 26 | 3.6 | 576 | 32 | 626 | 61 | 443 | 40 | <LOD | |
| Val | 1A | nmol * g FW ⁻¹ | 75 | 5.4 | 117 | 4.9 | 71 | 2.1 | 555 | 67 | 177 | 15 | 439 | 6.6 |
| Cystine | 1B | nmol * g FW ⁻¹ | <LOD | | <LOD | | <LOD | | <LOD | | <LOD | | 4.4 | 0.44 |
| Gly | 1B | nmol * g FW ⁻¹ | 323 | 41 | 618 | 36 | 184 | 7 | 427 | 40 | 183 | 23 | 282 | 26 |
| Met | 1B | nmol * g FW ⁻¹ | 54 | 2.9 | 54 | 3.1 | 28 | 1.0 | 72 | 9.6 | 73 | 8.2 | 44 | 3.5 |
| L-Ornithin | 1B | nmol * g FW ⁻¹ | 8.9 | 1.7 | 14 | 2.1 | 6.8 | 1.3 | 74 | 5.9 | 25 | 3.2 | 37 | 5.2 |
| Tryptamine | 1B | nmol * g FW ⁻¹ | 0.069 | 0.0059 | 0.064 | 0.011 | 0.17 | 0.016 | 0.87 | 0.14 | 0.66 | 0.22 | 0.31 | 0.037 |

| Citric acid | 1A | nmol * g FW ⁻¹ | 215 | 149 | 509 | 351 | <LOD | | | 36 ^b | 36 ^b | 769 ^b | 230 ^b | 45 ^b | 24 ^b | |
|---|----|---------------------------|-------|-------|-------|-------|-------|--------|-------|-----------------|-------------------|------------------|------------------|-----------------|-----------------|--|
| Butenedioic acid (fumaric acid & maleic acid) | 1A | nmol * g FW ⁻¹ | 315 | 23 | 256 | 26 | 231 | 21 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | | |
| Malic acid | 1A | Rel. quant. | 9083 | 681 | 10589 | 354 | 13706 | 1143 | 24762 | 4803 | 10502 | 606 | 198 | 39 | | |
| <i>a</i> -Ketoglutaric acid | 1B | nmol * g FW ⁻¹ | 484 | 39 | 486 | 30 | 519 | 36 | 1076 | 181 | 699 | 99 | 62 | 14 | | |
| Glucoronic acid | 1B | nmol * g FW ⁻¹ | 19 | 3.7 | 19 | 2.5 | 20 | 3.1 | 194 | 27 | 126 | 28 | 25 | 2.2 | | |
| Succinic acid | 1B | nmol * g FW ⁻¹ | 76 | 3.3 | 251 | 16 | 32 | 1.5 | 151 | 13 | 508 | 42 | 138 | 10 | | |
| Uric acid | 1B | nmol * g FW ⁻¹ | 26 | 7.6 | 49 | 26 | 23 | 3.8 | 6652 | 1017 | 1032 | 62 | 128 | 40 | | |
| Niacin | 1C | nmol * g FW ⁻¹ | <LOD | | <LOD | | <LOD | | <LOD | | 0.39 | 0.19 | 80 | 3.4 | | |
| Glucose | 1D | nmol * g FW ⁻¹ | 29509 | 1570 | 31340 | 3161 | 23496 | 2361 | 30752 | 5775 | 30029 | 13042 | 554 | 110 | | |
| Fructose | 1D | nmol * g FW ⁻¹ | 18430 | 1174 | 20918 | 1469 | 15802 | 1715 | 22741 | 2556 | 18569 | 6846 | 609 | 110 | | |
| Sucrose | 1D | nmol * g FW ⁻¹ | 7752 | 325 | 10697 | 687 | 11661 | 673 | 51782 | 7206 | 16210 | 2206 | 39925 | 4055 | | |
| Caffeoylputrescine | 1A | nmol * g FW ⁻¹ | 90 | 14 | 135 | 40 | 2349 | 183 | 1270 | 150 | 196 | 49 | <LOD | | | |
| Chlorogenic acid | 1A | nmol * g FW ⁻¹ | 881 | 85 | 971 | 73 | 1047 | 56 | 4365 | 792 | 9634 ^b | 803 ^b | 1117 | 199 | | |
| Rutin | 1A | nmol * g FW ⁻¹ | 1102 | 102 | 1436 | 128 | 1332 | 131 | 1819 | 242 | 275 | 47 | 341 | 54 | | |
| Quercetin | 1B | nmol * g FW ⁻¹ | 0.39 | 0.13 | 0.35 | 0.069 | 0.39 | 0.061 | 13 | 1.3 | 4.6 | 1.0 | <LOD | | | |
| Shikimic | 1B | nmol * g FW ⁻¹ | 134 | 26 | 137 | 15 | 102 | 14 | 437 | 76 | 366 | 38 | 17 | 3.0 | | |
| Nicotine | 1C | nmol * g FW ⁻¹ | 957 | 151 | 856 | 112 | 1467 | 216 | 11472 | 3629 | 5266 | 605 | 59 | 6.6 | | |
| Nornicotine | 1C | nmol * g FW ⁻¹ | 0.58 | 0.085 | 0.56 | 0.079 | 0.89 | 0.15 | 31 | 8.2 | 9.4 | 1.6 | <LOD | | | |
| Cotinine | 1C | nmol * g FW ⁻¹ | <LOD | | <LOD | | 0.020 | 0.0048 | <LOD | | 0.014 | 0.0039 | <LOD | | | |
| Anatabine | 1C | nmol * g FW ⁻¹ | 12 | 2.3 | 9.9 | 1.5 | 15 | 3.2 | 52 | 17 | 14 | 1.7 | <LOD | | | |
| Cinnamic acid | 2B | pmol * g FW ⁻¹ | 95 | 16 | 439 | 27 | 138 | 12 | <LOD | | 209 | 35 | 128 | 7.7 | | |
| <i>para</i> -Coumaric acid | 2B | pmol * g FW ⁻¹ | 229 | 23 | 326 | 13 | 160 | 13 | 121 | 16 | 295 | 97 | 319 | 30 | | |
| Caffeic acid | 2B | pmol * g FW ⁻¹ | 527 | 31 | 980 | 65 | 943 | 49 | 1363 | 161 | 7041 | 1719 | 1189 | 62 | | |
| Ferulic acid | 2B | pmol * g FW ⁻¹ | 27 | 2.1 | 67 | 3.7 | 22 | 1.5 | 275 | 29 | 1174 | 174 | 997 | 41 | | |
| Scopoletine | 2B | pmol * g FW ⁻¹ | 1.2 | 0.27 | 6.8 | 0.62 | 6.4 | 0.49 | 8.3 | 3.7 | 100 | 68 | 501 | 52 | | |
| Scopoline | 2B | Rel. quant. | 66 | 3.5 | 72 | 4.9 | 333 | 19 | 445 | 52 | 131 | 28 | 399 | 61 | | |

| | | | | | | | | | | | | | | |
|-------------------|----|---------------------------|-------|--------|-------|--------|-------|--------|------|-------|------|--------|------|-------|
| Coniferyl aldehyd | 2B | pmol * g FW ⁻¹ | 13 | 3.3 | 19 | 2.5 | 12 | 2.0 | 108 | 6.4 | 253 | 34 | 3670 | 437 |
| Sinapic acid | 2B | pmol * g FW ⁻¹ | 298 | 14 | 393 | 19 | 289 | 12 | 898 | 89 | 496 | 69 | 66 | 10 |
| Sinapyl aldehyd | 2B | Rel. quant. | 15 | 1.1 | 17 | 0.80 | 19 | 1.7 | 381 | 25 | 91 | 20 | 9.9 | 1.4 |
| ABA | 2A | pmol * g FW ⁻¹ | 251 | 18 | 533 | 22 | 408 | 26 | 4165 | 256 | 990 | 158 | 359 | 58 |
| SA | 2A | pmol * g FW ⁻¹ | 251 | 17 | 476 | 55 | 468 | 38 | 1377 | 66 | 269 | 24 | 580 | 54 |
| JA | 2A | pmol * g FW ⁻¹ | 6.2 | 2.7 | 13873 | 1202 | 16 | 4.5 | 93 | 32 | 3.7 | 2.4 | 1147 | 30 |
| JA-Ile | 2A | pmol * g FW ⁻¹ | <LOD | | 477 | 55 | <LOD | | <LOD | | <LOD | | 1245 | 79 |
| JA-Met | 2A | pmol * g FW ⁻¹ | <LOD | | 17 | 1.2 | <LOD | | <LOD | | <LOD | | <LOD | |
| JA-Val | 2A | pmol * g FW ⁻¹ | <LOD | | 53 | 1.8 | <LOD | | <LOD | | <LOD | | 26 | 3.7 |
| OH-JA | 2A | Rel. quant. | 88 | 37 | 6993 | 734 | 396 | 129 | 447 | 44 | 201 | 59 | 4884 | 350 |
| OH-JA-Ile | 2A | Rel. quant. | 5.2 | 1.4 | 2851 | 136 | 2.1 | 0.58 | 54 | 8.8 | 33 | 5.1 | 85 | 9.1 |
| COOH-JA-Ile | 2A | Rel. quant. | <LOD | | 1341 | 131 | 7.1 | 0.46 | 14 | 3.9 | 26 | 7.7 | 22 | 4.7 |
| IAA | 2B | pmol * g FW ⁻¹ | 69 | 4.9 | 63 | 1.9 | 37 | 1.1 | 48 | 5.8 | 272 | 60 | 67 | 6.1 |
| GA ₁ | 2B | pmol * g FW ⁻¹ | 0.84 | 0.58 | <LOD | | <LOD | | <LOD | | 1.1 | 1.1 | <LOD | |
| GA ₃ | 2B | pmol * g FW ⁻¹ | 2.3 | 0.48 | 0.83 | 0.17 | 2.3 | 0.56 | 6.3 | 1.2 | 6.5 | 3.1 | 27 | 10 |
| GA ₈ | 2B | pmol * g FW ⁻¹ | 80 | 5.2 | 107 | 5.6 | 67 | 6.9 | 40 | 3.9 | 114 | 14 | <LOD | |
| GA ₂₀ | 2B | pmol * g FW ⁻¹ | 29 | 4.0 | 39 | 5.0 | 2.6 | 1.3 | <LOD | | <LOD | | <LOD | |
| GA ₂₉ | 2B | pmol * g FW ⁻¹ | 234 | 17 | 283 | 13 | 276 | 21 | 243 | 77 | <LOD | | 19 | 19 |
| GA ₅₁ | 2B | pmol * g FW ⁻¹ | <LOD | | <LOD | | <LOD | | <LOD | | 2.3 | 0.84 | <LOD | |
| IP | 3 | pmol * g FW ⁻¹ | 0.061 | 0.0050 | 0.050 | 0.0039 | 0.049 | 0.0038 | 0.61 | 0.044 | 0.12 | 0.0061 | <LOD | |
| IPR | 3 | pmol * g FW ⁻¹ | 0.90 | 0.048 | 1.4 | 0.11 | 0.54 | 0.039 | 15 | 2.1 | 0.48 | 0.084 | <LOD | |
| tZ | 3 | pmol * g FW ⁻¹ | 1.2 | 0.13 | 0.91 | 0.047 | 1.1 | 0.077 | 0.97 | 0.094 | 0.36 | 0.076 | <LOD | |
| tZR | 3 | pmol * g FW ⁻¹ | 2.0 | 0.25 | 0.85 | 0.083 | 0.64 | 0.036 | 29 | 3.4 | 8.5 | 2.0 | 1.4 | 0.076 |
| cZ | 3 | pmol * g FW ⁻¹ | 0.069 | 0.010 | 0.072 | 0.015 | 0.15 | 0.027 | <LOD | | 0.44 | 0.071 | 0.52 | 0.073 |
| cZR | 3 | pmol * g FW ⁻¹ | 0.30 | 0.016 | 1.5 | 0.13 | 0.28 | 0.052 | 8.2 | 0.99 | 7.9 | 1.4 | 21 | 0.82 |
| DHZ | 3 | pmol * g FW ⁻¹ | 0.32 | 0.034 | 0.42 | 0.029 | 0.34 | 0.027 | <LOD | | 0.22 | 0.027 | <LOD | |
| DHZR | 3 | pmol * g FW ⁻¹ | 0.26 | 0.042 | 0.25 | 0.026 | 0.076 | 0.011 | 0.79 | 0.13 | 2.9 | 0.34 | 2.5 | 0.12 |
| cZOG | 3 | pmol * g FW ⁻¹ | 1.1 | 0.081 | 1.1 | 0.080 | 3.2 | 0.13 | 5.8 | 0.59 | 2.8 | 0.17 | <LOD | |
| DHZOG | 3 | pmol * g FW ⁻¹ | 0.42 | 0.11 | 0.38 | 0.082 | 0.31 | 0.061 | <LOD | | 1.2 | 0.26 | 3.0 | 2.0 |

| | | | | | | | | | | | | | | |
|---------------|---|---------------------------|-------|-------|------|-------|-------|-------|------|------|-------|-------|------|-------|
| <i>t</i> ZROG | 3 | pmol * g FW ⁻¹ | 1.3 | 0.079 | 1.2 | 0.089 | 1.1 | 0.061 | 76 | 14 | 16 | 2.5 | <LOD | |
| cZROG | 3 | pmol * g FW ⁻¹ | 2.8 | 0.18 | 3.1 | 0.34 | 5.9 | 0.29 | 73 | 2.7 | 22 | 2.1 | 0.64 | 0.039 |
| DHZROG | 3 | pmol * g FW ⁻¹ | 2.7 | 0.31 | 2.8 | 0.37 | 3.3 | 0.28 | 16 | 0.62 | 9.5 | 1.4 | <LOD | |
| <i>t</i> Z7G | 3 | pmol * g FW ⁻¹ | 5.0 | 0.19 | 4.8 | 0.13 | 6.8 | 0.21 | 6.6 | 0.52 | 7.1 | 0.91 | 0.19 | 0.035 |
| cZ7G | 3 | pmol * g FW ⁻¹ | 2.4 | 0.25 | 1.9 | 0.12 | 5.8 | 0.29 | 19 | 1.4 | 15 | 1.1 | 14 | 0.36 |
| DHZ7G | 3 | pmol * g FW ⁻¹ | 30 | 1.6 | 31 | 1.1 | 30 | 1.0 | 22 | 3.2 | 40 | 6.5 | 14 | 0.75 |
| DHZ9G | 3 | pmol * g FW ⁻¹ | 0.083 | 0.049 | 0.13 | 0.063 | 0.037 | 0.025 | <LOD | | 0.068 | 0.068 | <LOD | |

SE: Standard error (experiment 1, n=10; experiment 2, n≥6)

Rel. quant.: relative quantification based on the internal standards mentioned in Table S2-S8; without response factor

LOD: limit of detection

FW: fresh weight

^a Flower (already opened and likely pollinated) with removed corolla, stamen, style and pedicel

^b Relative quantification (based on the internal standards mentioned in Table S2-S8; without response factor) for citric acid in Experiment 2 and chlorogenic acid for Green capsules

Table S25 Response factors use for the analysis of flower and seed extracts.

| Analyte | Standard | Response factor ^a | | |
|--|--|------------------------------|---------------|-------|
| | | Flower base ^b | Green capsule | Seeds |
| Caffeoylputresine | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 0.30 | 0.34 | 0.38 |
| Chlorogenic acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 8.0 | - | 3.5 |
| Citric acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | - | - | - |
| Butenedioic acid (fumaric acid & maleic acid) | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 1.5 | 1.3 | 1.8 |
| Rutin | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 2.3 | 2.2 | 3.8 |
| Trp | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 4.3 | 3.3 | 4.2 |
| Tyramine | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 0.86 | 0.91 | 0.87 |
| α-Ketoglutaric acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 17 | 19 | 19 |
| Cystine | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 5.7 | 7.3 | 5.6 |
| Glucuronic acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 50 | 33 | 51 |
| Ornithine | ¹³ C ₆ , ¹⁵ N ₂ -Tyr | 2.9 | 3.6 | 2.4 |
| Quercetin | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 5.6 | 5.3 | 6.0 |
| Shikimic acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 43 | 91 | 50 |
| Succinic acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 1.4 | 2.2 | 1.6 |
| Tryptamine | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 0.15 | 0.14 | 0.19 |
| Uracil | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 6.3 | 8.6 | 6.8 |
| Uric acid | ¹³ C ₉ , ¹⁵ N ₁ -Tyr | 149 | 250 | 106 |
| Anabasine | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 3.6 | 2.4 | 4.3 |
| Anatabine | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 0.36 | 0.20 | 0.46 |
| Cotinine | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 0.11 | 0.056 | 0.13 |
| Niacin | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 4.8 | 5.0 | 6.5 |
| Nicotine | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 1.1 | 0.99 | 1.2 |
| Nornicotine | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 0.33 | 0.18 | 0.37 |
| Scopolamine | ¹³ C ₉ , ¹⁵ N ₁ -Phe | 0.12 | 0.078 | 0.14 |

| | | | | |
|-----------------------------|------------------------|-------|------|------|
| Cellubiose | Sorbitol | 6.1 | 5.1 | 5.5 |
| Fructose | Sorbitol | 0.71 | 0.64 | 0.68 |
| Glucose | Sorbitol | 0.95 | 0.79 | 0.90 |
| Rhamnose | Sorbitol | 1.4 | 1.1 | 1.3 |
| Sucrose | Sorbitol | 2.9 | 1.9 | 2.2 |
| JA-Met | D ₆ -JA-Ile | 2.4 | 1.9 | 2.4 |
| JA-Phe | D ₆ -JA-Ile | 2.1 | 2.5 | 1.7 |
| JA-Val | D ₆ -JA-Ile | 0.59 | 0.47 | 0.61 |
| Caffeic acid | 4-MU | 0.34 | 1.2 | 0.23 |
| Cinnamic acid | 4-MU | 5.2 | 6.4 | 6.2 |
| Coniferyl aldehyde | 4-MU | 0.34 | 0.37 | 0.41 |
| <i>para</i> -Coumaric acid | 4-MU | 0.14 | 0.25 | 0.17 |
| <i>meta</i> -Coumaric acid | 4-MU | 0.15 | 0.21 | 0.15 |
| <i>ortho</i> -Coumaric acid | 4-MU | 0.12 | 0.15 | 0.13 |
| Ferulic acid | 4-MU | 0.58 | 0.93 | 0.60 |
| Fraxetin | 4-MU | 0.35 | 1.2 | 0.37 |
| GA ₁ | 4-MU | 1.3 | 2.3 | 1.4 |
| GA ₃ | 4-MU | 0.83 | 1.9 | 1.4 |
| GA ₄ | 4-MU | 0.60 | 1.0 | 2.5 |
| GA ₅ | 4-MU | 3.4 | 5.6 | 4.0 |
| GA ₇ | 4-MU | 0.084 | 0.11 | 0.17 |
| GA ₈ | 4-MU | 16 | 88 | 18 |
| GA ₉ | 4-MU | 41 | 63 | 201 |
| GA ₁₂ | 4-MU | 20 | 48 | 30 |
| GA ₁₃ | 4-MU | 1.0 | 0.91 | 1.3 |
| GA ₂₀ | 4-MU | 7.2 | 9.7 | 10 |
| GA ₂₄ | 4-MU | 13 | 19 | 56 |
| GA ₂₉ | 4-MU | 40 | 212 | 25 |
| GA ₄₄ | 4-MU | 15 | 19 | 18 |

| | | | | |
|------------------|-----------------------|------|------|------|
| GA ₅₁ | 4-MU | 0.90 | 1.1 | 1.1 |
| IA-Ala | D ₅ -IAA | 0.51 | 0.45 | 0.73 |
| IAM | D ₅ -IAA | 0.57 | 0.70 | 0.72 |
| IBA | D ₅ -IAA | 2.1 | 3.4 | 3.2 |
| Scopoletine | 4-MU | 1.4 | 1.5 | 1.6 |
| Sinapic acid | 4-MU | 0.48 | 1.1 | 0.66 |
| <hr/> | | | | |
| BAP | D ₆ -IP | 2.5 | 2.2 | 2.7 |
| DHZ7G | D ₅ -tZ9G | 6.0 | 6.0 | 5.1 |
| DHZ9G | D ₅ -tZ9G | 2.4 | 2.4 | 2.2 |
| DHZOG | D ₅ -tZ9G | 6.4 | 6.2 | 6.6 |
| DHZROG | D ₃ -tZROG | 1.1 | 1.1 | 1.1 |
| IP9G | D ₆ -IP | 0.20 | 0.19 | 0.22 |

4-MU: 4-methylumbelliferone

^a Matrix and recovery corrected

^b Flower (already opened and likely pollinated) with removed corolla, stamen, style and pedicel