

An UPLC-MS/MS method for simultaneous identification and quantification of cell wall phenolics in *Brassica napus* seeds

Supporting Information

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Table S1 Source operation parameters applied for LC-MS and infusion experiments.

Parameters	Settings	
	LC-MS & –MS/MS experiments	Infusion experiments
Ion spray (IS) voltage	-2700 V	-4500 V
Nebulising gas (Gas 1)	N ₂ , 40 psi	N ₂ , 30 psi
Source temperature	450 °C (LIT-scans) or 550 °C (QqQ-scans)	room temperature (RT)
Drying gas (Gas 2)	N ₂ , 40 psi	0 psi
Curtain gas	N ₂ , 25 psi	N ₂ , 10 psi
Sample introduction flow-rate	150 µL/min	15 µL/min
Sample solvent	aq. MeOH / 0.01 NH ₄ OAc	aq. MeOH or MeCN / 0.01 – 0.1% NH ₄ OAc or 0.1% HCOOH

Table S2 Instrument settings applied for QqLIT- and QqQ-MS experiments.

Parameters	Settings	
	Enhanced MS (EMS) scan	Enhanced product ion (EPI) scan
Declustering potential (DP)		-20 V
Collision cell entrance potential (CEP)		-7 V
First quadrupole (Q1) resolution		Unit
Collision energy (CE) potential	-5 V	-20, -30, -35 or -40 V (with and without 15 V CE- spread)
Collision gas	N ₂ , 3 psi	N ₂ , 5 psi
Mass to charge ratio (<i>m/z</i>) range	100 – 1000	From 50
Third quadrupole (Q3) entry barrier	3 V	8 V
LIT scan rate		1000 or 4000 scans/s (filling times adjusted dynamically)

Table S3 Instrument settings applied for LC-QqTOF-MS experiments.

Parameters	Settings
Nebulizer gas	N ₂ , 1.4 bar
Drying gas	N ₂ , 6 L/min
Source temperature	190 °C
Capillary voltage	4000 V
End plate offset	-500 V
Funnel 1 RF	200 V _{pp}
Funnel 2 RF	200 V _{pp}
In-source collision potential	0 V
Hexapole RF	100 V _{pp}
Quadrupole ion energy	3 eV
Collision gas	N ₂
Collision energy	7 eV
Collision cell RF	150/350 V _{pp} (timing 50/50)
Transfer time	70 μs
Pre-pulse storage	5 μs
Pulser frequency	10 kHz
Spectra rate	3 Hz

Table S4 Intensities of selected analytes (expressed as heights of corresponding MS signals) obtained with ammonia acetate buffers of different molarities.

NH₄OAc concentration (mmol/L)	Compound ([M-H]⁻, <i>m/z</i>)	Peak areas (cps)^a			
		Caffeic acid (178.0)	Syringic acid (197.1)	Vanillic acid (167.0)	Vanillin (151.0)
13.0		1.3 E+07	8.8 E+06	8.2 E+06	2.0E+07
6.5		1.8 E+07	1.5 E+07	1.3 E+07	2.8E+07
3.2		2.3 E+07	1.8 E+07	1.4 E+07	3.5E+07
1.6		2.5 E+07	2.1 E+07	1.8 E+07	4.2E+07

^a The spectra (Q1 scans) were acquired by infusion of 300 μmol/L analyte solutions with a syringe pump (flow rate – 10 μL/min).

Table S5 Relative yields of alkali hydrolysis for individual phenylpropanoids obtained after saponification of 10 mg *Brasica napus* (cultivar Express) isolated cell wall material for 24 h either at 20 or 80 °C.

Phenylpropanoid	Label ^a	Analyte peak areas (cps) ± SD (RSD%) ^b		Intensity alteration (fold) ^c	Direction of alteration ^d	t-test ^e	Statistical significance ^e
		Hydrolysis at 20 °C	Hydrolysis at 80 °C				
Protocatehuic acid	1	3.21E+04 ± 1.19E+04 (37)	3.26E+06 ± 2.19E+05 (7)	101.6	↑	1.04E-07	++
4-hydroxybenzoic/salicylic acid	2, 3	6.87E+04 ± 1.20E+04 (18)	2.00E+05 ± 2.34E+04 (12)	2.9	↑	5.96E-05	++
2-(4-Hydroxyphenyl)ethanol	4	1.12E+02 ± 6.80E+01 (61)	3.26E+02 ± 1.34E+02 (41)	2.9	↑	2.94E-02	+
Orcinol (internal standard)	IS	1.90E+04 ± 2.38E+03 (13)	2.13E+04 ± 3.35E+03 (17)	1.2	↑	4.49E-01	n.s.
Vanillic acid	5	4.92E+03 ± 1.87E+03 (38)	2.57E+04 ± 2.57E+03 (10)	5.2	↑	1.26E-05	++
Caffeic acid	6	1.39E+04 ± 4.37E+03 (32)	2.05E+05 ± 9.98E+04 (49)	14.8	↑	8.72E-03	++
4-Hydroxybenzaldehyde	7	3.67E+04 ± 1.06E+04 (29)	1.50E+04 ± 4.56E+03 (30)	0.4	↓	9.43E-03	++
Syringic acid	8	2.50E+03 ± 5.91E+02 (24)	1.15E+04 ± 1.22E+03 (11)	4.6	↑	1.18E-05	++
5-Hydroxyferulic acid	9	2.38E+03 ± 1.27E+03 (53)	2.59E+04 ± 3.20E+03 (12)	10.9	↑	9.63E-06	++
Vanillin	10	5.68E+04 ± 1.48E+04 (26)	3.70E+04 ± 8.60E+03 (23)	0.7	↓	6.03E-02	n.s.
<i>p</i> -Coumaric acid	11	5.77E+04 ± 1.93E+04 (33)	2.23E+05 ± 2.74E+04 (12)	3.9	↑	6.37E-05	++
Benzoic acid	12	4.52E+04 ± 9.75E+03 (22)	6.08E+04 ± 6.55E+03 (11)	1.3	↑	3.82E-02	+
Coniferyl alcohol	13	b.d.l.	b.q.l.	–	n.a.	n.p.	n.s.
Syringaldehyde	14	4.41E+04 ± 9.22E+03 (21)	1.47E+04 ± 5.01E+03 (34)	0.3	↓	1.39E-03	++
Ferulic acid	15	2.93E+05 ± 7.17E+04 (24)	1.04E+06 ± 1.19E+05 (11)	3.5	↑	3.94E-05	++
Sinapylalcohol	16	b.q.l.	9.39E+02 ± 3.06E+02 (33)	n.a.	↑	n.p.	n.s.
Sinapic acid	17	1.12E+05 ± 7.92E+04 (71)	3.26E+05 ± 2.94E+04 (9)	2.9	↑	2.29E-03	++
Coniferylaldehyde	18	5.34E+03 ± 2.47E+03 (46)	1.60E+03 ± 3.19E+02 (20)	0.3	↓	2.41E-02	+
Sinapaldehyde	19	4.86E+02 ± 8.26E+00 (2)	5.07E+02 ± 6.36E+01 (13)	1.0	no alteration	5.42E-01	–

The semi-quantitative comparison (n = 4) of alkali hydrolysis yields was performed by UPLC-ESI-QqQ-MS/MS in MRM-mode on the basis of corresponding peak intensities. Peak intensities were calculated by integration of peak areas.

^a The phenylpropanoids are labeled according Table 1;

^b The Q1 and Q3 mass ranges (MRM-transitions) used for quantification are listed in Table 1;

^c Intensity alteration (of incubation temperature change from 20 to 80°C) was expressed as the ratio of peak areas integrated for the samples incubated at 80 and 20 °C

^d The peak intensity alteration values > 1.0 were interpreted as abundance increase, while those < 1.0 were considered as decrease when switching to incubation temperature of 80 °C

^e Statistical significance of differences was estimated by two-side Student t-test: p < 0.05 (+) or p < 0.01 (++)

b.d.l., below detection limit, b.q.l., below quantification limit, cps, counts per second; n.a., not analyzed; n.p., not performed; n.s., not significant; RSD%, relative standard deviation percentage; SD, standard deviation

Table S6 Analyte recoveries observed for individual phenylpropanoids in the sample preparation procedure under different time and temperature conditions^a.

Label ^b	Extraction recovery ^c (% ± SD%)	Analyte recoveries (% ± SD%) after saponification ^d of a solid standard ^e mixture for				Analyte recoveries (% ± SD%) after saponification ^d of a liquid standard ^f mixture for			
		24 h at 80 °C	24 h at 20 °C	15 h at 20 °C	4 h at 20 °C	24 h at 80 °C	24 h at 20 °C	15 h at 20 °C	4 h at 20 °C
1	63 ± 10	0 ± 0	0 ± 0	4 ± 1	37 ± 14	0 ± 0	0 ± 0	1 ± 0	18 ± 2
2 and 3	96 ± 5	105 ± 5	91 ± 4	93 ± 2	90 ± 8	100 ± 2	96 ± 3	93 ± 1	95 ± 2
4	99 ± 5	107 ± 15	97 ± 5	100 ± 2	104 ± 3	107 ± 3	95 ± 6	85 ± 6	93 ± 8
5	90 ± 1	127 ± 16	105 ± 3	112 ± 4	101 ± 5	117 ± 2	106 ± 5	102 ± 7	100 ± 3
6	104 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
7	117 ± 5	92 ± 7	80 ± 2	79 ± 2	79 ± 7	85 ± 2	75 ± 2	74 ± 4	77 ± 1
8	107 ± 2	65 ± 43	84 ± 56	118 ± 12	110 ± 7	95 ± 4	111 ± 11	102 ± 3	102 ± 4
10	88 ± 5	183 ± 22	126 ± 9	111 ± 13	97 ± 9	173 ± 4	116 ± 14	104 ± 7	106 ± 9
11	101 ± 3	96 ± 7	90 ± 4	96 ± 5	89 ± 8	93 ± 3	88 ± 3	89 ± 1	88 ± 3
12	86 ± 16	107 ± 22	89 ± 10	72 ± 12	91 ± 14	118 ± 4	91 ± 11	100 ± 12	106 ± 2
13	104 ± 8	1 ± 1	1 ± 0	0 ± 0	5 ± 8	1 ± 0	0 ± 0	0 ± 0	1 ± 1
14	110 ± 9	220 ± 23	142 ± 2	133 ± 11	111 ± 15	203 ± 24	125 ± 8	105 ± 11	99 ± 13
15	120 ± 7	80 ± 3	74 ± 9	85 ± 8	81 ± 4	78 ± 1	75 ± 11	74 ± 4	81 ± 4
16	55 ± 50	0 ± 0	1 ± 1	2 ± 0	2 ± 1	1 ± 0	2 ± 1	2 ± 0	1 ± 1
17	97 ± 9	17 ± 12	71 ± 19	88 ± 3	83 ± 20	36 ± 12	81 ± 16	92 ± 4	97 ± 15
18	100 ± 4	0 ± 0	34 ± 7	53 ± 6	70 ± 11	0 ± 0	37 ± 2	51 ± 3	71 ± 4
19	97 ± 1	0 ± 0	30 ± 5	46 ± 6	59 ± 13	0 ± 0	33 ± 6	50 ± 4	51 ± 4

All experiments were performed with a phenylpropanoid standard mixture (250 mmol/l per standard, n = 4)

^a The values are calculated on the basis of peak area data listed in Tables S6a and S6b; ^b The substances are labeled as shown in Table 1.

^c The recovery of ethyl acetate extraction was determined relative to the initial standard mixture (250 mmol/l per standard) dissolved in 80% aq. methanol: 200 µL of this mixture was added to 1800 µL water, thoroughly shaken and immediately repeatedly extracted with ethyl acetate (6 mL x 3) after adjusting pH to 3.0 with conc. HCl. The extracts were further treated as described in Materials and Methods part. This experiment can be referred to as extraction control.

^d The analyte recovery in saponification (alkaline hydrolysis) procedure was determined relative to extraction control.

^e The standard mixture (250 µmol/L per standard in 200 µL of 80% aq. methanol) was transferred to glass tubes and dried in nitrogen stream. Then 2000 µL of 1 mol/L NaOH was added and the tubes were treated at four time/temperature regimens as described in Materials and Methods part.

^f The standard mixture (250 µmol/L per standard in 200 µL of 80% aq. methanol) was transferred to glass tubes and 1800 µL of 1 mol/L NaOH was added. Then the tubes were treated at four time/temperature regimens as described in Materials and Methods part.

Table S6a Analyte peak areas observed in sample preparation experiments with saponification of solid standard mixture.

Label ^a	Analyte peak areas (cps) obtained in controls (n = 4)		Analyte peak areas (cps) obtained with a solid standard mixture ^d (n = 4)			
	Untreated control ^b	Extraction control ^c	24 h at 80 °C	24 h at 20 °C	15 h at 20 °C	4 h at 20 °C
1	3.23E+06 ± 2.17E+05	2.03E+06 ± 3.38E+05	1.90E+02 ± 2.41E+02	1.36E+03 ± 1.08E+03	7.84E+04 ± 2.27E+04	7.45E+05 ± 2.81E+05
2 and 3	6.42E+06 ± 5.24E+05	6.17E+06 ± 2.90E+05	6.47E+06 ± 2.90E+05	5.59E+06 ± 2.43E+05	5.76E+06 ± 1.10E+05	5.57E+06 ± 4.96E+05
4	7.48E+04 ± 2.00E+03	7.41E+04 ± 3.54E+03	7.93E+04 ± 1.09E+04	7.18E+04 ± 3.80E+03	7.48E+04 ± 1.97E+03	7.71E+04 ± 2.41E+03
5	2.75E+05 ± 8.33E+03	2.47E+05 ± 1.71E+03	3.14E+05 ± 3.87E+04	2.59E+05 ± 6.85E+03	2.76E+05 ± 8.92E+03	2.49E+05 ± 1.26E+04
6	4.36E+06 ± 2.17E+05	4.53E+06 ± 2.44E+05	4.56E+02 ± 3.74E+02	4.16E+03 ± 8.64E+02	6.32E+03 ± 1.51E+03	7.03E+03 ± 2.13E+03
7	9.30E+05 ± 3.67E+04	1.09E+06 ± 4.55E+04	1.00E+06 ± 8.04E+04	8.71E+05 ± 1.69E+04	8.60E+05 ± 1.80E+04	8.62E+05 ± 7.73E+04
8	2.09E+05 ± 9.85E+03	2.24E+05 ± 3.92E+03	1.46E+05 ± 9.72E+04	1.88E+05 ± 1.26E+05	2.70E+05 ± 2.42E+04	2.48E+05 ± 1.55E+04
10	4.62E+06 ± 1.83E+05	4.08E+06 ± 2.49E+05	7.48E+06 ± 9.10E+05	5.14E+06 ± 3.69E+05	4.46E+06 ± 4.33E+05	3.96E+06 ± 3.59E+05
11	5.10E+06 ± 1.50E+05	5.16E+06 ± 1.55E+05	4.96E+06 ± 3.46E+05	4.62E+06 ± 1.82E+05	4.93E+06 ± 2.22E+05	4.59E+06 ± 4.25E+05
12	5.39E+05 ± 4.73E+03	4.64E+05 ± 8.38E+04	4.98E+05 ± 1.03E+05	4.15E+05 ± 4.72E+04	3.15E+05 ± 5.98E+04	4.23E+05 ± 6.47E+04
13	2.79E+05 ± 4.03E+04	2.91E+05 ± 2.37E+04	1.97E+03 ± 1.73E+03	1.73E+03 ± 9.14E+02	4.90E+02 ± 2.17E+02	1.32E+04 ± 2.23E+04
14	2.18E+06 ± 6.43E+04	2.39E+06 ± 1.90E+05	5.27E+06 ± 5.59E+05	3.40E+06 ± 5.50E+04	3.14E+06 ± 2.36E+05	2.65E+06 ± 3.51E+05
15	1.88E+06 ± 1.34E+05	2.25E+06 ± 1.33E+05	1.81E+06 ± 5.97E+04	1.67E+06 ± 2.10E+05	1.90E+06 ± 1.48E+05	1.81E+06 ± 9.42E+04
16	1.51E+05 ± 8.00E+03	1.46E+05 ± 3.39E+04	3.01E+02 ± 2.71E+02	1.15E+03 ± 4.85E+02	1.51E+03 ± 1.99E+02	1.32E+03 ± 6.00E+02
17	7.12E+05 ± 7.28E+04	6.91E+05 ± 6.54E+04	1.20E+05 ± 8.29E+04	4.89E+05 ± 1.32E+05	5.74E+05 ± 7.29E+04	5.71E+05 ± 1.41E+05
18	6.38E+06 ± 1.69E+05	6.37E+06 ± 2.75E+05	2.04E+04 ± 1.33E+03	2.16E+06 ± 4.21E+05	3.37E+06 ± 3.18E+05	4.48E+06 ± 7.29E+05
19	3.58E+06 ± 4.25E+05	3.48E+06 ± 5.00E+04	1.45E+03 ± 8.78E+02	1.05E+06 ± 1.70E+05	1.62E+06 ± 1.74E+05	2.05E+06 ± 4.39E+05

^a The substances are labeled as shown in Table 1.

^b Initial standard mixture (250 mmol/l per standard dissolved in 80% aq. methanol) was directly analyzed by LC-QqQ-MS/MS without further sample preparation.

^c For extraction control, 200 µL of the initial standard mixture was added to 1800 µL water, thoroughly shaken and immediately repeatedly extracted with ethyl acetate (6 mL x 3) after adjusting pH to 3.0 with conc. HCl. The extracts were further treated as described in Materials and Methods part.

^d The standard mixture (250 µmol/L per standard in 200 µL of 80% aq. methanol) was transferred to glass tubes and dried in nitrogen stream. Then 2000 µL of 1 mol/L NaOH was added and the tubes were treated at four time/temperature regimens as described in Materials and Methods part.

Table S6b Analyte peak areas observed in sample preparation experiments with saponification of liquid standard mixture.

Label ^a	Analyte peak areas (cps) obtained in controls (n = 4)		Analyte peak areas (cps) obtained with a liquid standard mixture ^d (n = 4)			
	Untreated control ^b	Extraction control ^c	24 h at 80 °C	24 h at 20 °C	15 h at 20 °C	4 h at 20 °C
1	3.23E+06 ± 2.17E+05	2.03E+06 ± 3.38E+05	5.30E+01 ± 5.09E+01	4.16E+02 ± 5.33E+02	1.11E+04 ± 3.81E+03	3.59E+05 ± 3.58E+04
2 and 3	6.42E+06 ± 5.24E+05	6.17E+06 ± 2.90E+05	6.17E+06 ± 1.48E+05	5.91E+06 ± 1.92E+05	5.74E+06 ± 5.03E+04	5.89E+06 ± 1.37E+05
4	7.48E+04 ± 2.00E+03	7.41E+04 ± 3.54E+03	7.92E+04 ± 2.10E+03	7.05E+04 ± 4.44E+03	6.30E+04 ± 4.25E+03	6.92E+04 ± 5.75E+03
5	2.75E+05 ± 8.33E+03	2.47E+05 ± 1.71E+03	2.91E+05 ± 5.92E+03	2.63E+05 ± 1.34E+04	2.51E+05 ± 1.64E+04	2.48E+05 ± 8.10E+03
6	4.36E+06 ± 2.17E+05	4.53E+06 ± 2.44E+05	4.26E+02 ± 2.55E+02	2.01E+03 ± 8.13E+02	3.33E+03 ± 6.92E+02	4.44E+03 ± 5.93E+02
7	9.30E+05 ± 3.67E+04	1.09E+06 ± 4.55E+04	9.27E+05 ± 2.45E+04	8.20E+05 ± 2.48E+04	8.02E+05 ± 4.32E+04	8.37E+05 ± 1.53E+04
8	2.09E+05 ± 9.85E+03	2.24E+05 ± 3.92E+03	2.12E+05 ± 9.88E+03	2.48E+05 ± 2.47E+04	2.28E+05 ± 6.00E+03	2.29E+05 ± 9.78E+03
10	4.62E+06 ± 1.83E+05	4.08E+06 ± 2.49E+05	7.05E+06 ± 1.75E+05	4.73E+06 ± 5.87E+05	4.26E+06 ± 2.67E+05	4.30E+06 ± 3.82E+05
11	5.10E+06 ± 1.50E+05	5.16E+06 ± 1.55E+05	4.79E+06 ± 1.52E+05	4.52E+06 ± 1.34E+05	4.59E+06 ± 5.83E+04	4.52E+06 ± 1.44E+05
12	5.39E+05 ± 4.73E+03	4.64E+05 ± 8.38E+04	5.47E+05 ± 1.81E+04	4.20E+05 ± 5.10E+04	4.66E+05 ± 5.67E+04	4.92E+05 ± 1.14E+04
13	2.79E+05 ± 4.03E+04	2.91E+05 ± 2.37E+04	3.59E+03 ± 9.62E+02	1.22E+03 ± 6.81E+02	4.61E+02 ± 2.76E+02	2.83E+03 ± 1.95E+03
14	2.18E+06 ± 6.43E+04	2.39E+06 ± 1.90E+05	4.86E+06 ± 5.67E+05	3.00E+06 ± 1.81E+05	2.52E+06 ± 2.62E+05	2.37E+06 ± 3.22E+05
15	1.88E+06 ± 1.34E+05	2.25E+06 ± 1.33E+05	1.76E+06 ± 2.38E+04	1.68E+06 ± 2.56E+05	1.67E+06 ± 8.42E+04	1.83E+06 ± 9.22E+04
16	1.51E+05 ± 8.00E+03	1.46E+05 ± 3.39E+04	6.45E+02 ± 2.85E+02	1.57E+03 ± 7.60E+02	1.30E+03 ± 2.42E+02	1.55E+03 ± 4.25E+02
17	7.12E+05 ± 7.28E+04	6.91E+05 ± 6.54E+04	2.48E+05 ± 8.43E+04	5.56E+05 ± 1.13E+05	6.33E+05 ± 2.96E+04	6.72E+05 ± 1.01E+05
18	6.38E+06 ± 1.69E+05	6.37E+06 ± 2.75E+05	1.78E+04 ± 2.69E+03	2.36E+06 ± 1.22E+05	3.27E+06 ± 1.72E+05	4.50E+06 ± 2.36E+05
19	3.58E+06 ± 4.25E+05	3.48E+06 ± 5.00E+04	1.76E+03 ± 1.15E+03	1.14E+06 ± 2.00E+05	1.74E+06 ± 1.30E+05	1.78E+06 ± 1.56E+05

^a The substances are labeled as shown in Table 1.

^b Initial standard mixture (250 mmol/l per standard dissolved in 80% aq. methanol) was directly analyzed by LC-QqQ-MS/MS without further sample preparation.

^c For extraction control, 200 µL of the initial standard mixture was added to 1800 µL water, thoroughly shaken and immediately repeatedly extracted with ethyl acetate (6 mL x 3) after adjusting pH to 3.0 with conc. HCl. The extracts were further treated as described in Materials and Methods part. ^d The standard mixture (250 µmol/L per standard in 200 µL of 80% aq. methanol) was transferred to glass tubes and 1800 µL of 1 mol/L NaOH was added. Then the tubes were treated at four time/temperature regimens as described in Materials and Methods part.

Table S7 Unknown substances tentatively identified in saponified cell wall material of *Brassica napus* seeds.

Label ^{a,b}	t _R ^c	[M-H] ⁻ (m/z)	Fragment ions, m/z (%) ^d	MRM transition 1 (CE, eV) ^e	MRM transition 2 (CE, eV) ^e	Tentative assignment
Un 1	0.7 (V ₀)	169.0	97 (30), 107 (41), 110 (10), 123 (6), 125 (100), 151 (16), <u>169 (67)</u>	169.1 → 125.0 (20)	169.1 → 107.0 (20)	Gallic acid
Un 2	1.4	153.0	65 (3), 81 (7), 91 (13), 108 (44), 109 (100), <u>153 (11)</u>	153.0 → 109.0 (30)	153.0 → 108.0 (40)	2,3-Dihydroxybenzoic acid
Un 3*	1.8	195.0	93 (3), 106 (5), 107 (51), 109 (51), 123 (5), 135 (4), 151 (100), <u>195 (54)</u>	195.1 → 107.0 (30)	195.1 → 151.0 (20)	Carboxyhydroxy-phenylacetic acid
Un 4*	1.9	181.5	91 (3), 108 (16), 109 (6), 119 (6), 135 (15) 136 (18), 137 (100), 163 (18) <u>181 (100)</u>	181.1 → 137.0 (30)	181.1 → 108.1 (40)	Dihydrocaffeic acid
Un 5	2.7	123.0	81 (3), 93 (13), 95 (14), 108 (6), 122 (12), <u>123 (100)</u>	123.0 → 108.0 (30)	123.0 → 95.0 (30)	3,4-Dihydroxytoluene
Un 6	2.9	109.0	65 (5), 81 (3), 91 (15), 108 (41), <u>109 (100)</u>	109.0 → 91.0 (35)	109.0 → 108.0 (35)	Pyrocatechol
Un 7	3.1	125.0	69 (4), 79 (4), 97 (6), 107 (5), 124 (5), <u>125 (100)</u>	125.0 → 110.0 (20)	125.0 → 97.0 (20)	Pyrogallol
Un 8	3.4	107.1	65 (26), <u>107 (100)</u>	107.0 → 65.0 (35)		<i>p</i> -Cresol
Un 9	3.6	137.1	92 (5), 93 (3), 108 (16), 109 (4), 136 (17), <u>137 (100)</u>	137.1 → 108.0 (40)	137.1 → 92.0 (40)	1-(4-hydroxyphenyl)ethanol
Un 10	3.6	153.1	67 (4), 69 (6), 79 (11), 81 (3), 83 (8), 85(3), 95 (64), 97 (6), 107 (75), 108 (5), 109 (6), 123 (18), 124 (22), 125 (31), 151 (98), 152 (5), <u>153 (100)</u>	153.1 → 107.0 (35)	153.1 → 95.0 (35)	2-(3,4-dihydroxyphenyl)ethanol
Un 11	3.7	153.1	67 (2), 69 (3), 79 (2), 83 (2), 95 (17), 97 (2), 107 (19), 109 (2), 123 (7), 124 (6), 125 (14), 151 (43), <u>153 (100)</u>	153.1 → 107.1 (35)	153.1 → 95.1 (35)	Probably isomeric to 10
Un 12*	3.8	151.0	65 (3), 79 (2), 95 (11), 106 (6), 107 (100), 108 (5), 123 (6), 136 (5), <u>151 (73)</u>	151.0 → 107.0 (30)	151.0 → 136.1 (30)	4-Hydroxyphenylacetic acid
Un 13*	4.0	181.1	81 (2), 93(3), 108 (18), 109 (26), 117 (3), 134 (8), 135 (55), 136 (7), 137 (23), 145 (4), 163 (21), <u>181 (100)</u>	181.1 → 135.1 (30)	181.1 → 109.1 (30)	3-Hydroxydihydrocaffeyl aldehyde
Un 14	4.1	195.0	92 (11), 95 (3), 97 (4), 107 (31), 108 (29), 109 (22), 123 (16), 125 (10), 133 (7), 135 (34), 137 (34), 137 (6), 150 (6), 151 (100), 153 (10), 167 (3) 177 (2) <u>195 (87)</u>	195.1 → 107.0 (30)	195.1 → 151.1 (30)	3,4,5-Trihydroxycinnamic acid
Un 15*	4.5	163.0	107 (8), 119 (4), 133 (3), 134 (12), 135 (6), 145 (4), 161 (31), <u>163 (100)</u>	163.0 → 135.0 (35)		Caffeoyl aldehyde
Un 16	4.6	135.0	89 (9), 92 (1), 93 (1), 107 (2), 134 (40), <u>135 (100)</u>	135.1 → 134.1 (40)	135.1 → 89.0 (40)	3,4-dihydroxyethylenebenzene

Un 17	5.0	165.1	93 (28), 119 (33), 121 (100), 137 (22), 147 (11), <u>165 (40)</u>	165.1 → 121.1 (30)	165.1 → 137.0 (35)	Caffeoyl alcohol
Un 18	5.1	135.0	107 (77), <u>135 (100)</u>	135.1 → 107.0 (30)		2-(4-hydroxyphenyl)ethanal
Un 19	5.2	167.1	107 (9), 123 (11), 124 (10), 125 (21), 151 (12), <u>167 (100)</u>	167.1 → 125.0 (35)	167.1 → 151.0 (30)	3,4-Dihydroxy-5-methoxy-ethylbenzene
Un 20*	5.3	151.0	92 (6), 105 (6) 107 (28), 108 (41), 109 (16), 136 (13), <u>151 (100)</u>	151.0 → 108.0 (40)	151.0 → 109.0 (35)	3,4-Dihydroxyacetophenone
Un 21	5.6	179.1	107 (5), 108 (61), 109 (74), 135 (21), 137 (3), <u>179 (100)</u>	179.1 → 109.0 (35)	179.1 → 108.0 (40)	Guaiacylacetone
Un 22	5.7	167.1	95 (8), 109 (8), 121 (21), 123 (23) 124 (19), 125 (4), 137 (8), 139 (8), 149 (4), 152 (31), 165 (23), <u>167 (100)</u>	167.1 → 123.0 (20)		4-Methylsyringol
Un 23	5.7	135.0	92 (44), 93 (21), 120 (5), <u>135 (100)</u>	135.0 → 120.0 (30)	135.0 → 92.0 (40)	2-Methoxybenzaldehyde (or <i>p</i> -anisaldehyde)
Un 24	6.1	165.1	122 (100), 123 (52), 137 (32), 150 (20), 165 (74)	165.1 → 122.0 (30)	165.1 → 123.0 (35)	Acetovanillon
Un 25*	6.1	385.1	108 (6), 123 (3), 158 (8), 173 (10), 217 (5), 239 (6), 267 (68), 268 (5), 282 (48), 297 (6), 326 (22), 341 (100), 342 (6), <u>385 (11)</u>	385.1 → 341.3 (40)	385.1 → 267.2 (40)	Ferulic acid dimer 1
Un 26	6.1	167.0	108 (65), 123 (9), 152 (7), <u>167 (100)</u>	167.1 → 152.0 (20)		5-Hydroxyvanillin
Un 27*	6.1	193.1	89 (12), 117 (3), 133 (11), 134 (100), 135 (4), 149 (12) 178 (11), <u>193 (55)</u>	193.1 → 134.0 (20)	193.1 → 178.1 (20)	<i>cis</i> -Ferulic acid
Un 28	6.2	135.0	65 (3), 92 (24), 93 (8), <u>135 (100)</u>	135.0 → 92.1 (40)		4-Hydroxyacetophenone
Un 29*	6.5	149.1	121 (5), 134 (100), 135 (3), <u>149 (33)</u>	149.0 → 121.0 (20)		<i>p</i> -Vinylguaiacol
Un 30*	6.7	151.0	92 (3), 107 (95), 108 (6), 109 (4), 136 (10) <u>151 (100)</u>	151.0 → 107.0 (20)	151.0 → 135.9 (20)	Anisic acid
Un 31*	6.7	195.1	108 (4), 122 (6), 123 (5), 135 (6), 136 (35), 137 (6), 107 (3), 139 (5), 149 (5), 151 (90), 165 (6), 167 (4), 177 (13), 180 (5), <u>195 (100)</u>	195.1 → 151.1 (30)	195.1 → 136.1 (40)	3,5-Dimethoxyphenylacetic acid
Un 32	6.8	149.1	65 (19), 93 (9), 121 (50), 134 (18), <u>149 (100)</u>	149.0 → 121.1 (20)		4-Hydroxypropiophenone
Un 33	6.8	181.1	95 (4), 107 (3), 122 (24), 123 (9), 125 (9), 137 (50), 151 (7), 152 (7), 153 (6), 166 (5), <u>181 (100)</u>	181.1 → 137.0 (35)	181.1 → 122.0 (40)	Veratric acid
Un 34*	6.9	385.1	65 (5), 91 (6), 108 (25), 123 (13), 145 (22), 146 (16), 158 (64), 159 (100), 160 (7), 173 (29), 266 (27), 267 (11), 281 (25), 282 (22), 297 (7), 341 (3), <u>385 (47)</u>	385.1 → 159.1 (30)	385.1 → 281.3 (30)	Ferulic acid dimer 2
Un 35*	7.0	385.1	108 (13), 123 (4), 145 (8), 146 (10), 158 (18), 159 (35), 161 (4), 163 (4), 173 (10), 195 (6), 211 (10), 223 (10), 225 (8), 235 (21), 249 (8), 266 (15), 267 (100), 268 (15), 281 (25), 282 (75), 297 (52), 308 (8), 311 (10),	385.1 → 267.2 (30)	385.1 → 282.2 (30)	Ferulic acid dimer 3

			323 (13), 326 (48), 341 (69), <u>385 (71)</u>			
Un 36	7.0	195.1	109 (5), 136 (3), 137 (55), 151 (16), 165 (100), 179 (3), 180 (69), <u>195 (26)</u>	195.1 → 180.0 (20)	195.1 → 165.0 (30)	3,4-Dimethoxyphenylacetic acid
Un 37	7.1	165.1	122 (41), 123 (9), 150 (9), <u>165 (100)</u>	165.1 → 122.1 (35)	165.1 → 150.0 (35)	Veratraldehyde (?)
Un 38	7.1	301.0	59 (8), 109 (8), 137 (17), 161 (44), 211 (19), 229 (17), 257 (100), 283 (14), <u>301 (86)</u>	301.0 → 257.2 (20)		Ellagic acid
Un 39	7.2	153.1	109 (3), 111 (36), 123 (10), <u>153 (100)</u>	153.1 → 111.0 (30)	153.1 → 123.0 (40)	Unknown
Un 40*	7.8	385.1	91 (9), 108 (24), 109 (7), 123 (18), 134 (18), 145 (18), 146 (14), 149 (6), 158 (65), 159 (100), 173 (29), 178 (14), 193 (61), 239 (5), 250 (6), 266 (29), 267 (15), 281 (70), 282 (21), 297 (6), 313 (5), 341 (8), <u>385 (36)</u>	385.1 → 159.1 (30)	385.1 → 193.2 (30)	Ferulic acid dimer 4
Un 41	7.9	137.1	122 (9), 136 (18), <u>137 (100)</u>	137.1 → 122.0 (35)		Veratrole or 1,3-Dimethoxybenzene
Un 42*	7.9	195.1	95 (6), 107 (3), 109 (12), 123 (10), 135 (5), 137 (96), 138 (9), 151 (28), 152 (27), 153 (7), 165 (10), 167 (10), 180 (13), <u>195 (100)</u>	195.1 → 137.0 (40)	195.1 → 152.0 (30)	Acetosyringone
Un 43	8.5	135.0	91 (29), 135 (100)	135.0 → 91.0 (30)		Methylbenzoic acid
Un 44*	8.5	385.1	195 (4), 211 (6), 235 (4), 237 (6), 239 (22), 249 (7) 253 (6), 256 (11), 266 (10), 267 (59), 281 (19), 282 (31), 297 (10), 311 (11), 325 (6), 326 (39), 341 (74), 370 (6), <u>385 (100)</u>	385.1 → 341.2 (20)	385.1 → 267.3 (40)	Ferulic acid dimer 5
Un 45	8.6	305.1	88 (11), 93 (5), 107 (5), 121 (8), 149 (84), 163 (8), 164 (30), 193 (30), 195 (5), 208 (24), 217 (81), 223 (100), 245 (5), 261 (24), 287 (11), 305 (62)	305.1 → 223.1 (20)	305.1 → 149.0 (40)	Unknown sinapic acid conjugate
Un 46*	8.7	165.1	106 (11), 121 (79), 122 (4), <u>165 (100)</u>	165.1 → 121.1 (20)	165.1 → 106.0 (30)	3-Methoxyphenylacetic acid
Un 47*	9.8	445.1	164 (8), 181 (8), 203 (12), 223 (8), 255 (12), 269 (8), 297 (16), 327 (36), 342 (20), 355 (12), 357 (80), 401 (20), <u>445 (100)</u>	445.1 → 357.3 (30)	445.1 → 342.4 (30)	Sinapic acid dimer

Saponification (alkaline hydrolysis) of cell wall material (10 mg) was performed with 1 mol/L aq. NaOH for 24 h at 80 °C.

^a The substances are labeled in the order of their elution in 0.01% aq. NH₄CH₃COO / CH₃OH eluent system;

^b The species with assigned elemental composition are marked with asterisk;

^c Retention times observed in corresponding LC-ESI-QqLIT-EPI-TIC-chromatograms are listed;

^d Only the signals with relative intensities more than 2% are listed;

^e Collision energies are selected on the basis of several EPI and MRM runs under various Q2-collision conditions

Table S8 Relative yields of alkali hydrolysis for unknown substances obtained after saponification of 10 mg *Brasica napus* (cultivar Express) isolated cell wall material for 24 h either at 20 or 80 °C.

Label ^a	MRM-transition ^{b,c}	Analyte peak areas (cps) ± SD (RSD%)		Intensity alteration (fold) ^d	Direction of alteration ^e	t-test	Statistical significance ^f
		Hydrolysis at 20 °C	Hydrolysis at 80 °C				
Unknown 1	1	1.05E+03 ± 3.32E+02 (32)	1.91E+04 ± 8.62E+03 (45)	18.2	↑	5.76E-03	++
	2	1.48E+02 ± 5.80E+01 (39)	2.32E+03 ± 9.86E+02 (42)	15.7	↑	4.57E-03	++
Unknown 2	1	2.32E+02 ± 1.59E+02 (68)	1.76E+04 ± 1.09E+04 (62)	75.7	↑	1.88E-02	+
	2	b.q.l.	9.60E+03 ± 8.61E+02 (9)	–	↑	n.p.	quantified at 80°C
Unknown 3	1	b.q.l.	1.86E+03 ± 9.16E+02 (49)	–	↑	n.p.	quantified at 80°C
	2	b.d.l.	5.27E+03 ± 3.47E+03 (66)	–	↑	n.p.	detected at 80°C
Unknown 4	1	3.43E+04 ± 1.44E+04 (42)	2.83E+05 ± 9.58E+04 (34)	8.3	↑	2.14E-03	++
	2	4.64E+03 ± 3.75E+03 (81)	5.28E+04 ± 1.70E+04 (32)	11.4	↑	1.47E-03	++
Unknown 5	1	b.d.l.	1.46E+02 ± 7.85E+01 (54)	–	↑	n.p.	detected at 80°C
	2	b.d.l.	b.d.l.	–	n.a.	n.p.	detected at 80°C
Unknown 6	1	7.57E+01 ± 2.51E+01 (33)	2.40E+03 ± 1.52E+03 (63)	31.7	↑	2.21E-02	+
	2	b.q.l.	1.30E+03 ± 6.20E+02 (48)	–	↑	n.p.	quantified at 80°C
Unknown 7	1	b.q.l.	1.16E+03 ± 6.69E+02 (58)	–	↑	n.p.	quantified at 80°C
	2	8.81E+03 ± 3.99E+03 (45)	5.66E+04 ± 2.08E+04 (37)	6.4	↑	4.06E-03	++
Unknown 8	1	b.d.l.	2.74E+03 ± 8.02E+02 (29)	–	↑	n.p.	detected at 80°C
Unknown 9	1	2.85E+05 ± 8.98E+04 (32)	2.82E+05 ± 1.63E+05 (58)	1.0	n.a.	9.73E-01	not significant
	2	1.17E+05 ± 3.99E+04 (34)	1.16E+05 ± 7.02E+04 (60)	1.0	n.a.	9.84E-01	not significant
Unknown 10	1	1.54E+04 ± 1.07E+04 (69)	1.57E+05 ± 6.50E+04 (41)	10.2	↑	5.05E-03	++
	2	1.96E+04 ± 1.32E+04 (68)	1.88E+05 ± 7.72E+04 (41)	9.6	↑	5.16E-03	++
Unknown 11	1	1.71E+04 ± 1.13E+04 (66)	1.59E+05 ± 5.82E+04 (37)	9.3	↑	3.02E-03	++

	2	2.10E+04 ± 1.44E+04 (69)	1.96E+05 ± 8.40E+04 (43)	9.3	↑	6.37E-03	++
Unknown 12	1	4.72E+02 ± 6.28E+02 (133)	3.11E+03 ± 2.58E+03 (83)	6.6	↑	9.45E-02	not significant
	2	8.86E+01 ± 7.61E+01 (86)	5.36E+02 ± 1.13E+02 (21)	6.0	↑	6.05E-04	++
Unknown 13	1	1.72E+02 ± 4.07E+01 (24)	5.54E+02 ± 2.12E+02 (38)	3.2	↑	1.23E-02	+
	2	b.q.l.	1.25E+03 ± 2.81E+02 (22)	–	↑	n.p.	quantified at 80°C
Unknown 14	1	7.66E+02 ± 6.30E+02 (82)	5.55E+03 ± 1.82E+03 (33)	7.3	↑	2.55E-03	++
	2	b.q.l.	5.37E+03 ± 1.35E+03 (25)	–	↑	n.p.	quantified at 80°C
Unknown 15	1	b.d.l.	4.36E+03 ± 2.00E+02 (5)	–	↑	n.p.	detected at 80°C
Unknown 16	1	b.q.l.	1.59E+03 ± 8.35E+02 (52)	–	↑	n.p.	quantified at 80°C
	2	b.d.l.	8.43E+02 ± 4.46E+02 (53)	–	↑	n.p.	detected at 80°C
Unknown 17	1	b.d.l.	4.78E+02 ± 2.48E+02 (52)	–	↑	n.p.	detected at 80°C
	2	b.d.l.	8.15E+02 ± 6.85E+02 (84)	–	↑	n.p.	detected at 80°C
Unknown 18	1	b.d.l.	4.27E+03 ± 4.99E+02 (12)	–	↑	n.p.	detected at 80°C
Unknown 19	1	9.27E+02 ± 7.26E+02 (78)	6.74E+04 ± 2.23E+04 (33)	72.7	↑	1.01E-03	++
	2	9.27E+02 ± 5.60E+02 (60)	7.06E+04 ± 2.48E+04 (35)	76.2	↑	1.36E-03	++
Unknown 20	1	6.32E+02 ± 1.67E+02 (27)	5.23E+02 ± 2.40E+02 (46)	0.8	n.a.	4.87E-01	not significant
	2	b.d.l.	b.d.l.	–	↑	n.p.	not detected
Unknown 21	1	2.21E+04 ± 6.41E+03 (29)	2.11E+04 ± 9.29E+03 (44)	1.0	n.a.	8.55E-01	not significant
	2	2.43E+04 ± 6.34E+03 (26)	3.61E+04 ± 1.20E+04 (33)	1.5	n.a.	1.32E-01	not significant
Unknown 22	1	b.q.l.	2.95E+02 ± 4.42E+01 (15)	–	↑	n.p.	quantified at 80°C
Unknown 23	1	2.93E+03 ± 8.21E+02 (28)	3.79E+04 ± 5.74E+03 (15)	12.9	↑	1.98E-05	++
	2	9.58E+03 ± 1.95E+03 (20)	1.41E+05 ± 2.04E+04 (14)	14.7	↑	1.40E-05	++
Unknown 24	1	1.67E+02 ± 1.58E+02 (95)	1.04E+04 ± 2.46E+03 (24)	62.1	↑	1.68E-04	++
	2	b.d.l.	4.01E+03 ± 5.02E+02 (13)	–	↑	n.p.	detected at 80°C

Unknown 25	1	2.61E+02 ± 1.33E+02 (51)	3.56E+03 ± 6.38E+02 (18)	13.6	↑	5.42E-05	++
	2	9.38E+02 ± 3.98E+02 (42)	1.83E+04 ± 2.11E+03 (11)	19.5	↑	3.49E-06	++
Unknown 26	1	8.50E+02 ± 2.73E+02 (32)	2.73E+03 ± 6.53E+02 (24)	3.2	↑	1.79E-03	++
	1	9.68E+04 ± 2.94E+04 (30)	2.00E+05 ± 6.00E+04 (30)	2.1	↑	2.12E-02	+
Unknown 27	2	1.50E+04 ± 4.78E+03 (32)	3.31E+04 ± 8.88E+03 (27)	2.2	↑	1.14E-02	+
	1	b.d.l.	b.d.l.	–	n.a.	n.p.	not detected
Unknown 29	1	1.87E+03 ± 1.55E+03 (83)	4.35E+03 ± 1.60E+03 (37)	2.3	↑	6.78E-02	not significant
Unknown 30	1	2.29E+03 ± 1.39E+03 (61)	1.79E+04 ± 1.57E+03 (9)	7.8	↑	5.82E-06	++
	2	b.q.l.	4.01E+03 ± 1.50E+03 (37)	–	↑	n.p.	quantified at 80°C
Unknown 31	1	b.q.l.	b.q.l.	–	n.a.	n.p.	not quantified
	2	b.q.l.	b.q.l.	–	n.a.	n.p.	not quantified
Unknown 32	1	2.61E+03 ± 1.88E+03 (72)	7.88E+03 ± 1.09E+03 (14)	3.0	↑	2.82E-03	++
Unknown 33	1	b.q.l.	2.73E+03 ± 4.80E+02 (18)	–	↑	n.p.	quantified at 80°C
	2	b.d.l.	1.08E+03 ± 5.65E+02 (53)	–	↑	n.p.	detected at 80°C
Unknown 34	1	3.69E+02 ± 1.92E+02 (52)	5.65E+04 ± 1.76E+04 (31)	153.0	↑	7.02E-04	++
	2	3.40E+02 ± 1.03E+02 (30)	3.37E+04 ± 7.36E+03 (22)	98.9	↑	1.02E-04	++
Unknown 35	1	1.24E+03 ± 4.06E+02 (33)	1.69E+04 ± 3.26E+03 (19)	13.6	↑	7.50E-05	++
	2	8.01E+02 ± 2.93E+02 (37)	2.48E+04 ± 4.80E+03 (19)	31.0	↑	5.86E-05	++
Unknown 36	1	1.94E+03 ± 2.58E+01 (1)	2.73E+04 ± 3.74E+03 (14)	14.0	↑	1.01E-05	++
	2	2.20E+03 ± 4.48E+02 (20)	3.23E+04 ± 4.33E+03 (13)	14.7	↑	8.94E-06	++
Unknown 37	1	1.25E+03 ± 3.64E+02 (29)	8.46E+03 ± 1.50E+03 (18)	6.8	↑	8.43E-05	++
	2	b.q.l.	4.24E+02 ± 8.82E+01 (21)	–	↑	n.p.	quantified at 80°C
Unknown 38	1	b.q.l.	b.q.l.	–	n.a.	n.p.	not quantified
Unknown 39	1	b.q.l.	2.61E+03 ± 6.11E+02 (23)	–	↑	n.p.	quantified at 80°C

	2	b.q.l.	2.24E+03 ± 1.21E+03 (54)	–	↑	n.p.	quantified at 80°C
Unknown 40	1	2.59E+02 ± 4.79E+01 (18)	1.99E+04 ± 4.81E+03 (24)	76.8	↑	1.82E-04	++
	2	8.16E+03 ± 2.48E+03 (30)	2.67E+04 ± 4.12E+03 (15)	3.3	↑	2.51E-04	++
Unknown 41	1	1.58E+02 ± 9.55E+01 (60)	1.56E+03 ± 4.47E+02 (29)	9.8	↑	8.71E-04	++
Unknown 42	1	b.d.l.	b.q.l.	–	n.a.	n.p.	not quantified
	2	b.d.l.	4.03E+02 ± 3.09E+02 (77)	–	↑	n.p.	quantified at 80°C
Unknown 43	1	3.25E+04 ± 3.03E+03 (9)	5.12E+04 ± 2.18E+03 (4)	1.6	↑	5.77E-05	++
Unknown 44	1	1.07E+03 ± 5.39E+02 (51)	1.22E+04 ± 2.75E+02 (2)	11.5	↑	2.64E-08	++
	2	1.11E+03 ± 5.46E+02 (49)	1.39E+04 ± 3.30E+02 (2)	12.5	↑	1.63E-08	++
Unknown 45	1	5.96E+03 ± 2.78E+03 (47)	2.20E+04 ± 3.11E+03 (14)	3.7	↑	2.55E-04	++
	2	4.02E+03 ± 1.77E+03 (44)	1.25E+04 ± 2.01E+03 (16)	3.1	↑	7.18E-04	++
Unknown 46	1	b.d.l.	2.23E+04 ± 3.24E+03 (15)	–	↑	n.p.	quantified at 80°C
	2	b.d.l.	4.32E+03 ± 5.39E+02 (12)	–	↑	n.p.	quantified at 80°C
Unknown 47	1	1.96E+04 ± 1.13E+04 (58)	1.16E+03 ± 1.23E+02 (11)	0.1	↓	1.72E-02	+
	2	7.70E+03 ± 4.24E+03 (55)	4.59E+02 ± 5.04E+01 (11)	0.1	↓	1.43E-02	+

Saponification (alkaline hydrolysis) of cell wall material (n = 4) was performed with 1 mol/L aq. NaOH for 24 h either at 20 or 80 °C. The semi-quantitative comparison of alkali hydrolysis yield was performed by UPLC-ESI-QqQ-MS/MS in MRM mode on the basis of corresponding peak intensities. Peak intensities were calculated by integration of peak areas.

^a The unknowns are labeled according Table S7; ^b Corresponding Q1 and Q3 mass ranges (MRM-transitions) are listed in Table S7; ^c Only the peaks demonstrated previously annotated retention times are considered here; ^d Intensity alteration (of incubation temperature change from 20 to 80 °C) was expressed as the ratio of peak areas integrated for the samples incubated at 80 and 20°C; ^e The peak intensity alteration values > 1.0 were interpreted as increase, while those < 1.0 were considered as decrease; ^f Significance of differences was estimated by two-side Student t-test: p < 0.05 (+) or p < 0.01 (++)

b.d.l., below detection limit; b.q.l, below quantification limit; cps, counts per second; n.a., not analyzed; n.p., not performed; RSD%, relative standard deviation percentage; SD, standard deviation

Table S9 Sensitivity and linearity parameters obtained for phenolic standards by external and internal method standardization.

Label ^a	External calibration ^b						Internal calibration ^{c,d}					
	LOD _i (mol)	LOQ _i (mol)	LDR _i	Slope	Intercept	R ²	LOD _m (mol)	LOQ _m (mol)	LDR _m	Slope	Intercept	R ²
1	5.0E-14	5.0E-14	1.0E+04	2.16E+04	-6.53E+02	0.997	1.0E-12	5.0E-11	≥ 2.0E+01	5.04E-03	4.96E-02	0.994
2	5.0E-17	5.0E-5	5.0E+04	3.90E+04	1.33E+03	0.999	2.5E-16	5.0E-14	2.0E+04	2.28E-01	8.87E-03	0.975
3	5.0E-15	5.0E-15	1.0E+03	1.15E+05	1.36E+03	0.997	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	1.0E-16	1.0E-16	1.0E+04	6.96E+02	-1.62E+00	0.996	2.5E-14	1.0E-13	5.0E+03	5.28E-03	-4.44E-04	0.984
IS	5.0E-14	1.0E-13	2.0E+03	3.23E+02	5.78E+01	0.994	–	–	–	–	–	–
5	5.0E-14	1.0E-13	1.0E+04	1.75E+03	1.08E+02	0.999	2.5E-15	1.0E-13	2.5E+03	1.13E-02	1.61E-03	0.993
6	1.0E-15	5.0E-14	2.0E+03	3.57E+04	1.31E+03	0.993	2.5E-15	5.0E-12	≥ 2.0E+02	5.68E-04	-3.53E-03	0.935
7	2.5E-14	5.0E-14	5.0E+02	5.64E+03	2.30E+02	0.992	5.0E-17	1.0E-13	1.0E+04	2.20E-02	1.28E-02	0.994
8	1.0E-14	2.5E-13	2.0E+03	1.53E+03	1.16E+02	0.997	2.5E-15	5.0E-14	5.0E+03	1.08E-02	2.93E-04	0.993
9	1.0E-14	2.5E-14	2.0E+04	1.59E+04	-1.07E+01	0.998	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	5.0E-17	1.0E-15	1.0E+05	3.44E+04	3.92E+02	0.998	1.0E-16	2.5E-14	4.0E+04	2.34E-01	2.31E-02	0.987
11	1.0E-16	5.0E-15	2.0E+04	3.72E+04	6.85E+02	0.997	1.0E-16	2.5E-14	4.0E+04	2.32E-01	7.67E-04	0.992
12	2.5E-14	2.5E-13	2.0E+03	4.48E+03	1.41E+03	0.996	5.0E-15	2.5E-12	1.0E+02	1.37E-02	3.1E-01	0.960
13	2.5E-14	2.5E-14	4.0E+04	2.21E+03	4.95E+01	0.996	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14	2.5E-15	2.5E-14	1.0E+04	1.43E+04	2.11E+02	0.997	5.0E-16	2.5E-14	4.0E+04	1.36E-01	1.53E-03	0.982
15	2.5E-17	5.0E-15	1.0E+05	1.42E+04	2.67E+02	0.991	5.0E-15	5.0E-14	2.0E+04	1.07E-01	-2.40E-03	0.990
16	1.0E-13	2.5E-13	4.0E+03	1.41E+03	1.41E+02	0.992	1.0E-11	1.0E-11	≥ 1.0E+02	6.85E-05	-6.03E-04	0.960
17	2.5E-17	2.5E-14	2.0E+04	5.03E+03	2.39E+02	0.994	1.0E-13	5.0E-12	5.0E+01	2.07E-02	-1.43E-01	0.956
18	5.0E-17	2.5E-15	4.0E+04	5.36E+04	8.31E+02	0.991	5.0E-17	2.5E-14	1.0E+04	1.82E-01	3.67E-03	0.990
19	5.0E-17	2.5E-15	4.0E+04	2.79E+04	2.62E+02	0.996	1.0E-15	1.0E-13	1.0E+04	6.69E-02	-7.02E-03	0.985

All parameters were determined in three independent dilution series; ^a The phenolics are labeled as in Table 1; ^b calculated with untreated standards; ^c calculated with saponified standards: the standard mixtures dissolved in 80% CH₃OH were dried in the flow of nitrogen and treated with 1 mol/L aq. NaOH for 15 h at 20 °C; ^d internal standard (2.2 μmol/L 5-methylresorcinol) was added to ethyl acetate before re-extraction of phenylpropanoids from hydrolysates

Table S10 Relative sensitivity^a and linearity parameters calculated for selected unknowns in saponified cell wall material, obtained from *Brassica napus* (cultivar Express) seeds.

Label ^b	Sensitivity parameters ^c		Linearity parameters ^c			
	LOD ^d (dilution factor)	LOQ ^e (dilution factor)	Linear dynamic ^f range (up to dilution factor)	Slope	Intercept	R ²
Un 4	16	8	1	8.34E+02	8.37	0.940
Un 6	8	4	1	1.15E+03	-70.90	0.983
Un 8	16	8	1	2.49E+03	-9.57	0.981
Un 10	256	64	1	4.95E+04	-173.00	0.999
Un 13	32	16	1	1.40E+04	422.00	0.983
Un 14	4	4	1	2.30E+04	-3.32E+03	0.939
Un 15	32	16	1	2.21E+03	34.90	0.977
Un 19	64	32	1	6.94E+03	-18.60	0.989
Un 21	256	128	8	2.48E+04	209.00	0.989
Un 23	256	32	1	4.77E+04	6.07E+03	0.995
Un 27	4098	1024	1	2.01E+05	116.00	0.995
Un 30	32	32	1	1.04E+04	135.00	0.991
Un 36	256	128	1	3.46E+04	77.40	0.995
Un 42	128	64	1	6.05E+03	34.40	0.992
Un 43	256	128	1	3.96E+04	437.00	0.999

Selected unknowns represent species demonstrating widely varying intensities in saponified cell wall preparations.

^a Sensitivities are expressed relative to the analyte intensities in undiluted preparation;

^b The species are labeled according to Table S7;

^c The parameters were determined in three independent dilution series obtained from one pooled preparation with dilution increment of 2x;

^d LOD (limit of detection) was defined as a dilution factor resulting in sample signal intensity/blank signal intensity ≥ 3 in all three parallel dilution series;

^e LOQ (limit of quantification) was defined as a dilution factor resulting in sample signal intensity/blank signal intensity ≥ 10 in all three parallel dilution series;

^f the lowest dilution factor (the highest analyte intensity) within observed analyte linear dynamic range

Table S11 Validation of LC-MS/MS method with intraday and inter-day precision values determined for identified phenolics and selected unknowns

Label ^a	Intraday precision (n = 5)		Inter-day precision (n = 5/day) ^b	
	t _R (min) ± SD (RSD%)	S _a (counts) ± SD (RSD%)	t _R (min) ± SD (RSD%)	S _a (counts) ± SD (RSD%)
1	1.9 ± 0.0 (1)	2.14E+06 ± 4.72E+04 (2)	1.9 ± 0.0 (1)	2.13E+06 ± 1.33E+05 (6)
2 and 3	3.2 ± 0.0 (0)	1.84E+05 ± 5.17E+03 (3)	3.2 ± 0.0 (0)	1.88E+05 ± 1.40E+04 (7)
IS	4.3 ± 0.0 (0)	2.97E+06 ± 9.00E+04 (3)	4.3 ± 0.0 (0)	2.91E+06 ± 1.03E+05 (4)
4	4.2 ± 0.1 (2)	2.20E+02 ± 2.22E+01 (10)	4.2 ± 0.1 (2)	2.21E+02 ± 6.58E+01 (30)
5	4.3 ± 0.0 (0)	6.18E+04 ± 2.90E+03 (5)	4.3 ± 0.0 (0)	6.12E+04 ± 5.52E+03 (9)
6	4.5 ± 0.0 (0)	6.36E+04 ± 3.05E+03 (5)	4.5 ± 0.0 (0)	6.35E+04 ± 3.65E+03 (6)
7	4.6 ± 0.0 (0)	1.82E+04 ± 7.85E+02 (4)	4.6 ± 0.0 (0)	1.90E+04 ± 1.81E+03 (10)
8	4.9 ± 0.0 (0)	1.03E+04 ± 3.78E+02 (4)	4.9 ± 0.0 (0)	9.72E+03 ± 6.73E+02 (7)
9	5.2 ± 0.0 (0)	2.75E+03 ± 1.88E+02 (7)	5.2 ± 0.0 (0)	2.75E+03 ± 1.92E+02 (7)
10	5.7 ± 0.0 (0)	5.95E+04 ± 2.04E+03 (3)	5.7 ± 0.0 (0)	5.89E+04 ± 2.48E+03 (4)
11	5.7 ± 0.0 (0)	9.94E+04 ± 4.91E+03 (5)	5.7 ± 0.0 (0)	1.01E+05 ± 4.42E+03 (4)
12	5.8 ± 0.0 (0)	7.94E+04 ± 2.12E+03 (3)	5.8 ± 0.0 (0)	7.52E+04 ± 7.64E+03 (10)
14	6.4 ± 0.0 (0)	2.80E+04 ± 7.60E+02 (3)	6.4 ± 0.0 (0)	2.62E+04 ± 2.55E+03 (10)
15	6.4 ± 0.0 (0)	9.29E+05 ± 8.04E+04 (9)	6.4 ± 0.0 (0)	8.92E+05 ± 8.55E+04 (10)
16	6.7 ± 0.0 (0)	6.99E+02 ± 1.23E+02 (18)	6.7 ± 0.0 (0)	8.00E+02 ± 1.96E+02 (24)
17	6.7 ± 0.0 (0)	2.35E+05 ± 2.23E+04 (9)	6.7 ± 0.0 (0)	2.44E+05 ± 2.44E+04 (10)
19	7.6 ± 0.1 (1)	1.23E+02 ± 2.12E+01 (17)	7.6 ± 0.0 (1)	1.14E+02 ± 3.11E+01 (27)
Un 4	1.9 ± 0.0 (2)	6.37E+03 ± 2.42E+02 (4)	1.9 ± 0.0 (2)	6.35E+03 ± 6.71E+02 (11)
Un 6	2.9 ± 0.0 (1)	4.92E+03 ± 2.10E+02 (4)	2.9 ± 0.0 (1)	4.90E+03 ± 2.47E+02 (5)
Un 8	3.4 ± 0.0 (1)	3.74E+03 ± 2.10E+02 (6)	3.4 ± 0.0 (1)	3.52E+03 ± 2.47E+02 (7)
Un 10	3.6 ± 0.0 (0)	2.86E+04 ± 9.07E+02 (3)	3.6 ± 0.0 (0)	2.89E+04 ± 1.10E+03 (4)
Un 13	4.0 ± 0.0 (0)	2.27E+04 ± 1.30E+03 (6)	4.0 ± 0.0 (0)	2.31E+04 ± 1.90E+03 (8)
Un 14	3.4 ± 0.0 (0)	6.65E+03 ± 5.40E+02 (8)	3.4 ± 0.0 (1)	6.58E+03 ± 5.46E+02 (8)
Un 15	4.5 ± 0.0 (0)	2.03E+03 ± 1.11E+02 (5)	4.5 ± 0.0 (0)	2.11E+03 ± 3.11E+02 (15)
Un 19	5.2 ± 0.0 (0)	4.51E+03 ± 2.90E+02 (6)	5.2 ± 0.0 (0)	4.68E+03 ± 4.04E+02 (9)
Un 21	5.6 ± 0.0 (0)	2.49E+04 ± 3.35E+03 (13)	5.6 ± 0.0 (0)	2.36E+04 ± 3.43E+03 (15)
Un 23	5.7 ± 0.0 (0)	5.79E+04 ± 1.91E+03 (3)	5.7 ± 0.0 (0)	5.79E+04 ± 2.70E+03 (5)
Un 27	6.0 ± 0.0 (0)	1.28E+05 ± 4.32E+03 (3)	6.0 ± 0.0 (0)	1.24E+05 ± 8.33E+03 (7)
Un 30	6.5 ± 0.0 (0)	1.04E+04 ± 8.82E+02 (8)	6.5 ± 0.0 (0)	9.53E+03 ± 1.35E+03 (14)
Un 36	7.0 ± 0.0 (0)	3.37E+04 ± 3.16E+03 (9)	7.0 ± 0.0 (0)	3.00E+04 ± 3.43E+03 (11)
Un 42	7.9 ± 0.0 (0)	3.47E+03 ± 8.85E+01 (3)	7.9 ± 0.0 (0)	3.18E+03 ± 2.27E+02 (7)
Un 43	8.4 ± 0.0 (0)	3.38E+04 ± 1.48E+03 (4)	8.4 ± 0.0 (0)	3.23E+04 ± 2.16E+03 (7)

Validation was performed with a pooled saponified cell wall preparation obtained from the seeds of *Brassica napus* (cultivar Express).

^a Compounds are labeled as in Tables 1 and S7;

^b An inter-day precision was determined on five consecutive days;

SD, standard deviation; RSD%, relative standard deviation percentage

Table S12 The content of esterified hydroxycinnamates and hydroxybenzoates in purified cell wall material isolated from *Brassica napus* seeds of different cultivars.

Label ^a	The content of esterified ^d phenolics (pmol/mg cell wall material)		
	Express (n = 4)	Drakkar (n = 4)	Lisora (n = 4)
1	868.5 ± 261.8 (30)	1096.0 ± 807.9 (74)	685.1 ± 641.4 (94)
2	6.5 ± 0.3 (5)	4.2 ± 2.0 (48)	5.2 ± 1.8 (35)
3 ^b	2.4 ± 0.3 (11)	1.6 ± 0.7 (43)	1.8 ± 0.4 (24)
4	3.3 ± 1.2 (35)	7.9 ± 5.0 (63)	2.8 ± 0.9 (32)
5	25.9 ± 5.7 (22)	23.5 ± 4.8 (20)	25.1 ± 4.4 (18)
6	552.5 ± 291.7 (53)	414.0 ± 102.3 (25)	498.0 ± 240.9 (48)
7	55.8 ± 2.0 (3)	46.5 ± 8.4 (18)	60.9 ± 10.6 (17)
8	13.9 ± 2.2 (16)	12.3 ± 3.8 (31)	13.2 ± 1.2 (9)
9 ^b	3.9 ± 1.1 (29)	4.4 ± 1.1 (25)	5.9 ± 1.6 (27)
10	26.9 ± 2.7 (10)	24.6 ± 4.1 (17)	48.5 ± 6.9 (14)
11	20.4 ± 3.0 (15)	17.9 ± 2.6 (14)	31.3 ± 4.3 (14)
12 ^b	215.6 ± 27.4 (13)	137.6 ± 59.1 (43)	170.1 ± 8.5 (5)
13 ^c	b.d.l.	b.d.l.	b.d.l.
14	16.5 ± 1.9 (11)	20.4 ± 3.7 (18)	25.5 ± 5.6 (22)
15	353.5 ± 40.6 (11)	238.9 ± 66.2 (28)	368.0 ± 72.3 (20)
16	812.0 ± 303.4 (37)	488.0 ± 129.9 (27)	702.0 ± 164.5 (23)
17	1380.5 ± 83.4 (6)	1253.5 ± 75.5 (6)	1369.0 ± 128.0 (9)
18	2.8 ± 0.6 (20)	4.1 ± 0.9 (21)	9.1 ± 3.0 (32)
19	3.9 ± 0.2 (6)	5.4 ± 0.2 (3)	6.4 ± 0.9 (14)

^a The phenylpropanoids are labeled as in Table 1; ^b The substances were standardized externally; ^c The concentration of the substance was below its detection limit determined by both external and internal calibration strategies; ^d Saponification was performed with 1 mol/L NaOH for 15 h at 20 °C; b.d.l., below detection limit

Table S13 The peak areas of unknown substances observed in LC-MS/MS experiments with saponified cell wall material isolated from *Brasica napus* seeds (cultivars Express, Drakkar and Lisora).

Label ^a	Tr ^{b,c}	Analyte peak areas (cps) ± SD (RSD%) ^c		
		Express (n = 4)	Drakkar (n = 4)	Lisora (n = 4)
Un 1	1	1.06E+04 ± 5.39E+03 (51)	4.16E+03 ± 1.18E+03 (28)	1.36E+04 ± 8.26E+03 (61)
Un 1	2*	1.78E+03 ± 7.85E+02 (44)	8.71E+02 ± 3.30E+02 (38)	2.21E+03 ± 9.77E+02 (44)
Un 2	1**	2.08E+04 ± 9.18E+03 (44)	2.58E+03 ± 1.82E+03 (71)	2.18E+04 ± 5.01E+03 (23)
Un 2	2*/**	7.14E+03 ± 5.19E+03 (73)	6.79E+02 ± 9.10E+02 (134)	8.86E+03 ± 3.13E+03 (35)
Un 3	1	b.d.l	b.d.l	b.d.l
Un 3	2	4.14E+03 ± 1.94E+03 (47)	b.d.l	7.17E+03 ± 6.23E+03 (87)
Un 4	1**	5.93E+05 ± 9.65E+04 (16)	2.20E+05 ± 1.20E+05 (54)	5.39E+05 ± 9.44E+04 (18)
Un 4	2**	1.04E+05 ± 1.26E+04 (12)	3.91E+04 ± 2.21E+04 (56)	9.70E+04 ± 1.74E+04 (18)
Un 5	1	1.58E+02 ± 5.95E+01 (38)	b.q.l.	b.q.l.
Un 5	2	b.d.l	b.d.l	b.d.l
Un 6	1	3.87E+03 ± 5.16E+03 (133)	b.d.l	3.38E+03 ± 5.31E+03 (157)
Un 6	2	1.98E+03 ± 2.69E+03 (136)	b.d.l	1.77E+03 ± 2.81E+03 (159)
Un 7	1	8.70E+02 ± 8.12E+02 (93)	5.41E+02 ± 1.52E+02 (28)	4.24E+02 ± 3.09E+02 (73)
Un 7	2	1.09E+04 ± 4.81E+03 (44)	b.d.l.	5.96E+03 ± 1.88E+03 (32)
Un 8*/**		3.64E+03 ± 1.87E+03 (51)	7.17E+02 ± 2.67E+02 (37)	4.21E+03 ± 2.13E+03 (51)
Un 9	1	1.90E+06 ± 1.24E+05 (7)	9.46E+05 ± 5.14E+05 (54)	1.59E+06 ± 1.93E+05 (12)
Un 9	2	7.39E+05 ± 4.40E+04 (6)	3.75E+05 ± 2.03E+05 (54)	6.12E+05 ± 8.09E+04 (13)
Un 10	1*	1.97E+04 ± 1.00E+04 (51)	2.08E+03 ± 8.68E+02 (42)	2.38E+04 ± 1.26E+04 (53)
Un 10	2*	2.38E+04 ± 1.44E+04 (61)	1.67E+03 ± 8.19E+02 (49)	2.89E+04 ± 1.65E+04 (57)
Un 11	1*	2.03E+04 ± 1.25E+04 (62)	1.65E+03 ± 5.45E+02 (33)	2.31E+04 ± 1.29E+04 (56)
Un 11	2*	2.46E+04 ± 1.36E+04 (55)	1.76E+03 ± 8.56E+02 (49)	3.08E+04 ± 1.74E+04 (57)
Un 12	1	5.44E+03 ± 2.40E+04 (44)	9.08E+03 ± 2.76E+03 (30)	7.99E+03 ± 1.94E+03 (24)
Un 12	2	1.70E+03 ± 1.64E+03 (96)	1.32E+03 ± 9.19E+02 (69)	3.89E+03 ± 4.12E+03 (106)
Un 13	1*/**	5.06E+02 ± 1.07E+02 (21)	1.37E+02 ± 1.13E+02 (83)	2.90E+02 ± 6.78E+01 (23)
Un 13	2*	6.69E+02 ± 2.19E+02 (33)	2.66E+02 ± 1.96E+02 (74)	6.74E+02 ± 2.57E+02 (38)
Un 14	1	7.93E+03 ± 2.37E+03 (30)	7.96E+03 ± 4.28E+03 (54)	7.99E+03 ± 3.17E+03 (40)
Un 14	2	5.72E+03 ± 1.38E+03 (24)	2.89E+03 ± 1.69E+03 (58)	1.77E+04 ± 2.09E+04 (118)
Un 15**		4.34E+03 ± 7.20E+02 (17)	9.06E+02 ± 5.58E+02 (62)	4.17E+03 ± 7.77E+02 (19)
Un 16	1	1.98E+03 ± 1.41E+03 (71)	b.d.l.	2.61E+03 ± 1.50E+03 (57)
Un 16	2	1.98E+03 ± 1.31E+03 (66)	b.d.l.	1.63E+03 ± 1.23E+03 (75)
Un 17	1*	2.70E+03 ± 2.22E+03 (82)	4.89E+03 ± 1.58E+03 (32)	1.81E+03 ± 8.59E+02 (47)
Un 17	2	5.11E+02 ± 3.67E+02 (72)	2.32E+02 ± 6.59E+01 (28)	8.55E+02 ± 2.12E+02 (25)
Un 18**		4.08E+03 ± 1.44E+03 (35)	2.27E+03 ± 9.26E+02 (41)	4.74E+03 ± 8.78E+02 (19)
Un 19	1*/**	1.14E+04 ± 6.78E+03 (59)	1.81E+03 ± 1.42E+03 (79)	1.40E+04 ± 3.99E+03 (29)
Un 19	2*/**	1.12E+04 ± 6.53E+03 (58)	6.03E+02 ± 7.20E+02 (119)	1.20E+04 ± 4.12E+03 (34)
Un 20	1	1.66E+03 ± 8.35E+02 (50)	1.07E+03 ± 9.49E+02 (88)	2.20E+03 ± 6.24E+02 (28)
Un 20	2	b.d.l.	b.d.l.	b.d.l
Un 21	1*	3.08E+04 ± 7.83E+03 (25)	5.19E+03 ± 1.10E+03 (21)	4.02E+04 ± 1.77E+04 (44)
Un 21	2**	5.79E+04 ± 1.62E+04 (28)	1.98E+04 ± 7.28E+03 (40)	7.18E+04 ± 2.01E+04 (28)
Un 22		6.86E+02 ± 5.71E+02 (83)	4.22E+02 ± 2.36E+02 (56)	8.42E+02 ± 6.70E+02 (80)
Un 23	1*/**	3.79E+04 ± 4.80E+03 (13)	2.17E+04 ± 5.18E+03 (24)	3.56E+04 ± 6.83E+03 (19)
Un 23	2**	1.42E+05 ± 1.09E+04 (8)	7.97E+04 ± 1.49E+04 (19)	1.35E+05 ± 2.54E+04 (19)
Un 24	1*/**	4.04E+03 ± 1.41E+03 (35)	1.13E+03 ± 9.48E+02 (84)	4.46E+03 ± 8.01E+02 (18)
Un 24	2	1.46E+03 ± 3.98E+02 (27)	b.d.l.	1.70E+03 ± 7.06E+02 (42)

Un 25	1	3.94E+03 ± 1.24E+03 (32)	1.06E+03 ± 4.05E+02 (38)	3.53E+03 ± 1.26E+03 (36)
Un 25	2	2.22E+04 ± 6.48E+03 (29)	5.53E+03 ± 1.45E+03 (26)	1.98E+04 ± 6.80E+03 (34)
Un 26		2.78E+03 ± 5.70E+02 (20)	1.74E+03 ± 9.92E+02 (57)	2.86E+03 ± 4.11E+02 (14)
Un 27	1*	3.30E+05 ± 1.56E+05 (47)	1.12E+05 ± 4.51E+04 (40)	4.20E+05 ± 2.51E+05 (60)
Un 27	2*	5.64E+04 ± 2.96E+04 (52)	1.78E+04 ± 7.27E+03 (41)	6.78E+04 ± 4.34E+04 (64)
Un 28		b.d.l.	b.d.l.	b.d.l.
Un 29**		8.42E+03 ± 3.24E+03 (38)	5.26E+03 ± 1.12E+03 (21)	9.29E+03 ± 3.38E+03 (36)
Un 30	1 ^{*/**}	1.05E+04 ± 2.37E+03 (23)	5.92E+03 ± 1.30E+03 (22)	1.02E+04 ± 1.43E+03 (14)
Un 30	2*	9.53E+03 ± 2.94E+03 (31)	3.97E+03 ± 2.75E+03 (69)	9.60E+03 ± 1.61E+03 (17)
Un 31	1	b.d.l.	b.d.l.	b.d.l.
Un 31	2	b.d.l.	b.d.l.	b.d.l.
Un 32		9.50E+03 ± 2.32E+03 (24)	5.58E+03 ± 2.10E+03 (38)	6.33E+03 ± 1.38E+03 (22)
Un 33	1	7.69E+02 ± 6.59E+02 (86)	b.d.l.	6.83E+02 ± 5.96E+02 (87)
Un 33	2	7.44E+02 ± 5.22E+02 (70)	b.d.l.	7.00E+02 ± 4.89E+02 (70)
Un 34	1**	4.23E+04 ± 1.43E+04 (34)	1.04E+04 ± 5.70E+03 (55)	3.94E+04 ± 9.78E+03 (25)
Un 34	2**	2.56E+04 ± 8.56E+03 (34)	6.79E+03 ± 3.66E+03 (54)	2.34E+04 ± 5.85E+03 (25)
Un 35	1	b.d.l.	b.d.l.	b.d.l.
Un 35	2	b.d.l.	b.d.l.	b.d.l.
Un 36	1	1.90E+04 ± 3.00E+03 (16)	1.68E+04 ± 5.97E+03 (35)	2.08E+04 ± 7.76E+03 (37)
Un 36	2	2.33E+04 ± 5.35E+03 (23)	1.99E+04 ± 6.92E+03 (35)	2.58E+04 ± 9.39E+03 (36)
Un 37	1 ^{*/**}	1.59E+04 ± 7.80E+03 (49)	4.14E+03 ± 2.49E+03 (60)	1.08E+04 ± 2.49E+03 (23)
Un 37	2	3.95E+03 ± 1.38E+03 (35)	6.16E+03 ± 4.35E+03 (71)	2.11E+03 ± 1.33E+03 (63)
Un 38		b.d.l.	b.d.l.	b.d.l.
Un 39	1*	2.05E+03 ± 1.43E+03 (70)	5.59E+02 ± 4.58E+02 (82)	3.83E+03 ± 1.32E+03 (34)
Un 39	2	1.38E+03 ± 9.52E+02 (69)	6.50E+02 ± 3.84E+02 (59)	1.86E+03 ± 6.16E+02 (33)
Un 40	1 ^{*/**}	2.99E+04 ± 9.27E+03 (31)	7.65E+03 ± 3.28E+03 (43)	3.94E+04 ± 1.96E+04 (50)
Un 40	2**	2.58E+04 ± 6.85E+03 (27)	7.75E+03 ± 2.04E+03 (26)	2.28E+04 ± 5.59E+03 (24)
Un 41*		1.07E+02 ± 5.74E+01 (54)	3.25E+01 ± 1.49E+01 (46)	1.32E+02 ± 5.82E+01 (44)
Un 42	1	2.29E+03 ± 1.83E+03 (80)	b.d.l.	3.49E+03 ± 1.96E+03 (56)
Un 42	2	1.48E+03 ± 1.05E+03 (71)	b.d.l.	2.49E+03 ± 1.21E+03 (48)
Un 43		4.78E+04 ± 5.91E+03 (12)	4.09E+04 ± 2.65E+03 (6)	4.70E+04 ± 3.52E+03 (8)
Un 44	1**	1.11E+04 ± 3.59E+03 (32)	3.00E+03 ± 1.47E+03 (49)	1.27E+04 ± 3.44E+03 (27)
Un 44	2**	1.22E+04 ± 4.20E+03 (34)	3.29E+03 ± 1.37E+03 (42)	1.29E+04 ± 3.56E+03 (28)
Un 45	1 ^{*/**}	1.32E+04 ± 1.86E+03 (14)	8.59E+03 ± 1.40E+03 (16)	1.14E+04 ± 1.68E+03 (15)
Un 45	2**	8.13E+03 ± 1.69E+03 (21)	4.51E+03 ± 8.32E+02 (18)	6.05E+03 ± 1.14E+03 (19)
Un 46	1*	1.89E+04 ± 6.30E+03 (33)	9.49E+03 ± 6.90E+03 (73)	2.62E+04 ± 9.65E+03 (37)
Un 46	2*	4.08E+03 ± 1.46E+03 (36)	1.91E+03 ± 1.17E+03 (61)	4.98E+03 ± 1.96E+03 (39)
Un 47	1**	3.18E+02 ± 1.38E+02 (43)	8.95E+02 ± 1.60E+02 (18)	3.39E+02 ± 1.71E+01 (5)
Un 47	2**	1.16E+02 ± 6.18E+01 (53)	4.08E+02 ± 8.40E+01 (21)	1.44E+02 ± 3.50E+01 (24)

Saponification was performed with 1 mol/L NaOH for 24 h at 80 °C

^a The unknowns are labeled according Table S7; ^b Corresponding Q1 and Q3 mass ranges (MRM-transitions) are listed in Table S7; ^c Only the peaks demonstrated previously annotated retention times are considered here

b.d.l., below detection limit; b.q.l, below quantification limit; cps, counts per second; RSD%, relative standard deviation percentage; SD, standard deviation; Tr, MRM-transition

*, difference significant ($p < 0.05$); **, difference significant ($p < 0.01$)

Table S14 Alterations in intensities of unknown substances observed in LC-MS/MS experiments with saponified cell wall material isolated from *Brasica napus* seeds (cultivars Express, Drakkar and Lisora).

Inter-cultivar paired comparison of compound intensities (n = 4)							
Label ^a	Tr ^{b,c}	Express/ Drakkar		Express/Lisora		Drakkar/Lisora	
		Alteration (fold)	t-test	Alteration (fold)	t-test	Alteration (fold)	t-test
Un 1	2	2.0	0.0755	0.8	0.5275	0.4	0.0414*
Un 2	1	8.1	0.0081**	1.0	0.8510	0.1	0.0004**
	2	10.5	0.0498*	0.8	0.5893	0.1	0.0024**
Un 4	1	2.7	0.0029**	1.1	0.4542	0.4	0.0058**
	2	2.7	0.0023**	1.1	0.5601	0.4	0.0062**
Un 8		5.1	0.0215*	0.9	0.6993	0.2	0.0173*
Un 10	1	9.4	0.0128*	0.8	0.6263	0.1	0.0137*
	2	14.2	0.0220*	0.8	0.6529	0.1	0.0164*
Un 11	1	12.3	0.0246*	0.9	0.7615	0.1	0.0158*
	2	13.9	0.0156*	0.8	0.5937	0.1	0.0158*
Un 13	1	3.7	0.0033**	1.7	0.0147*	0.5	0.0594
	2	2.5	0.0337*	1.0	0.9739	0.4	0.0447*
Un 15		4.8	0.0003**	1.0	0.7591	0.2	0.0005**
Un 17	1	0.6	0.1595	1.5	0.4837	2.7	0.0141*
Un 18		1.8	0.0785	0.9	0.4635	0.5	0.0082**
Un 19	1	6.3	0.0322*	0.8	0.5377	0.1	0.0012**
	2	18.7	0.0176*	0.9	0.8610	0.1	0.0016**
Un 21	1	3.1	0.0138*	0.8	0.3724	0.2	0.0231*
	2	3.9	0.0051**	0.8	0.3215	0.2	0.0027**
Un 23	1	1.7	0.0037**	1.1	0.5936	0.6	0.0176*
	2	1.8	0.0005**	1.1	0.6187	0.6	0.0094**
Un 24	1	3.6	0.0140*	0.9	0.6242	0.3	0.0017**
Un 25	1	3.7	0.0045**	1.1	0.6631	0.3	0.0096**
	2	4.0	0.0024**	1.1	0.6311	0.3	0.0064**
Un 27	1	3.0	0.0364*	0.8	0.5661	0.3	0.0521
	2	3.2	0.0444*	0.8	0.6801	0.3	0.0635
Un 30	1	1.8	0.0146*	1.0	0.7996	0.6	0.0047**
	2	2.4	0.0327*	1.0	0.9657	0.4	0.0123*
Un 34	1	4.1	0.0061**	1.1	0.7514	0.3	0.0022**
	2	3.8	0.0069**	1.1	0.6860	0.3	0.0030**
Un 37	1	5.1	0.0218*	1.5	0.2552	0.3	0.0068**
Un 35	1	3.4	0.0062**	0.8	0.4781	0.2	0.0043**
	2	4.5	0.0005**	1.1	0.6454	0.2	0.0018**
Un 39	1	3.7	0.0939	0.7	0.4172	0.2	0.0391*
Un 40	1	3.9	0.0040**	0.8	0.4166	0.2	0.0189*
	2	3.3	0.0024**	1.1	0.5325	0.3	0.0023**
Un 41		3.3	0.0460*	0.8	0.5614	0.2	0.0162*
Un 44	1	3.7	0.0059**	0.9	0.5527	0.2	0.0021**
	2	3.7	0.0068**	0.9	0.8200	0.3	0.0024**
Un 45	1	1.5	0.0076**	1.2	0.2117	0.8	0.0411*
	2	1.8	0.0084**	1.3	0.0864	0.7	0.0713
Un 46	1	2.0	0.0910	0.7	0.2507	0.4	0.0304*
	2	2.1	0.0597	0.8	0.4898	0.4	0.0360*
Un 47	1	0.4	0.0016**	0.9	0.7779	2.6	0.0005**
	2	0.3	0.0014**	0.8	0.4645	2.8	0.0011**

Saponification was performed with 1 mol/L NaOH for 24 h at 80 °C

^a The unknowns are labeled according Table S7; ^b Corresponding Q1 and Q3 mass ranges (MRM-transitions) are listed in Table S7; ^c Only the peaks demonstrated previously annotated retention times are considered here
b.d.l., below detection limit; b.q.l, below quantification limit; Tr, MRM-transition
*, alteration significant ($p < 0.05$); **, alteration significant ($p < 0.01$)

Table S15 Lignin contents ($\mu\text{g}/\text{mg}$ fresh weight) in mature seeds of three different *Brassica napus* cultivars.

<i>B. napus</i> cultivar	Lignin content ($\mu\text{g}/\text{mg}$ fresh seed weight) \pm SD (RSD%)
Express	54.1 \pm 4.3 (8)
Lisora	70.9 \pm 15.3 (22)
Drakkar	13.0 \pm 3.7 (28)

Lignin contents (n = 4) were determined by the lignothyoglycolic acid method

Figures

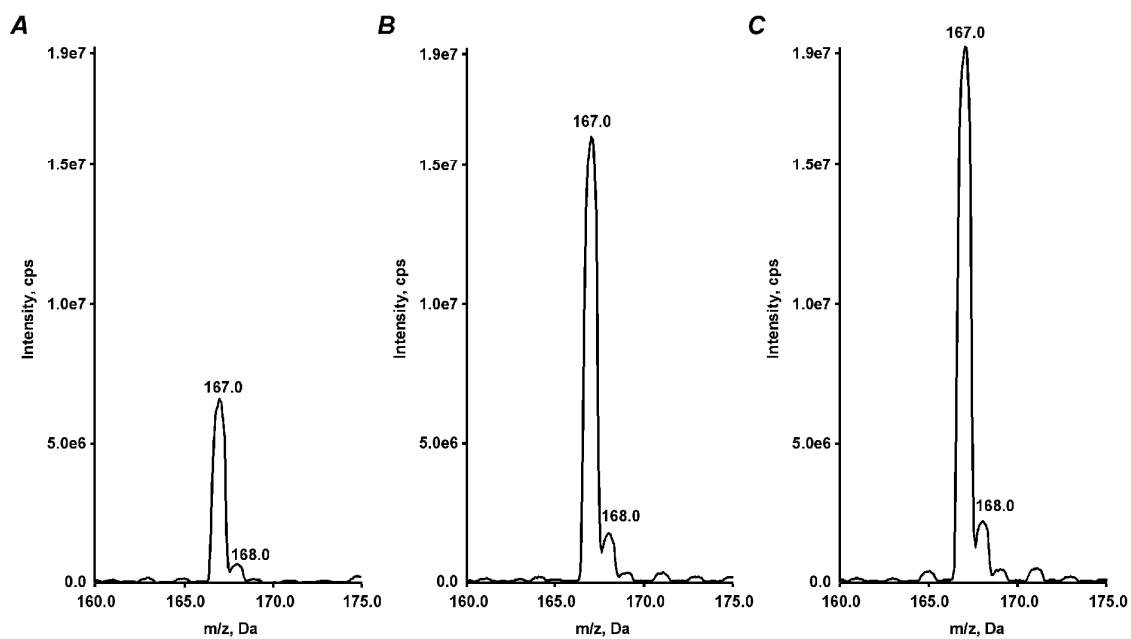


Fig S1 Comparison of MS signal intensities obtained in Q1 scans performed with vanillic acid using 0.1% formic acid (pH 3.0) in 60% aq. acetonitrile (Panel A), 0.1% ammonium acetate (pH 6.8) in 60% aq. acetonitrile (Panel B) or 0.1% ammonium acetate (pH 6.8) in 60% aq. methanol (Panel C) as solvents.

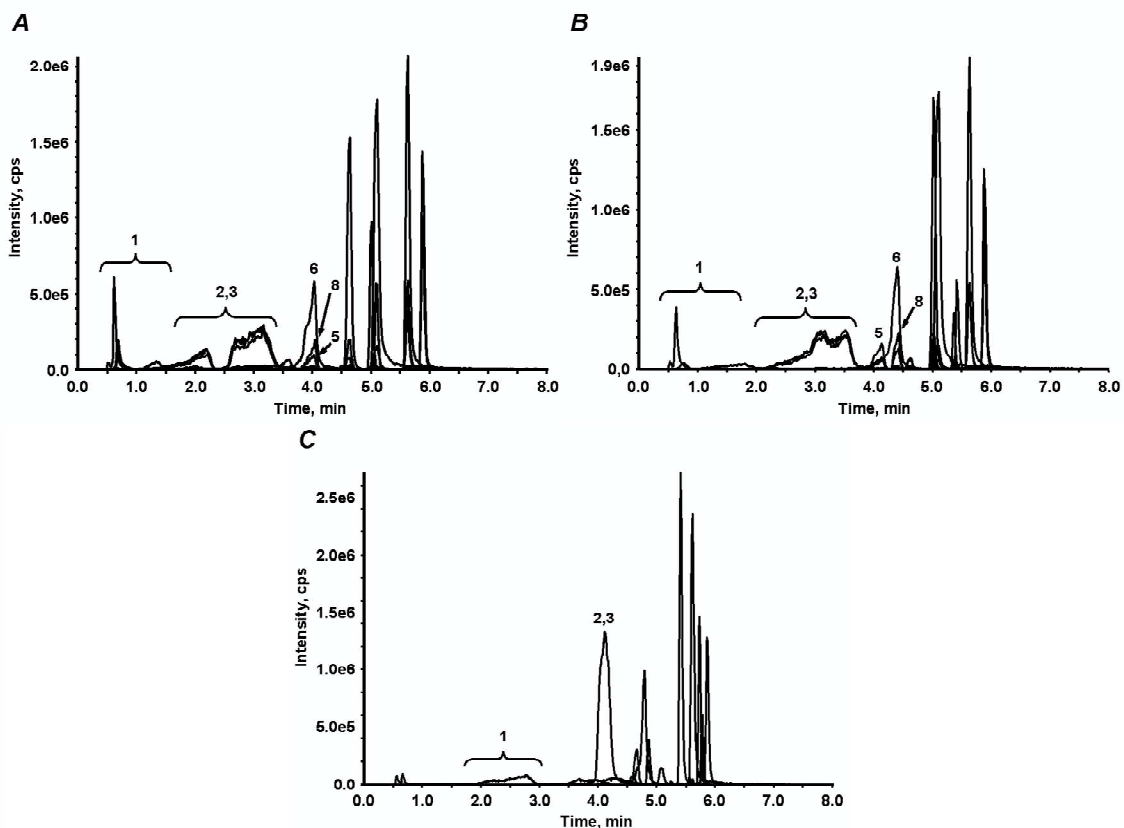


Fig S2 RP-UPLC-MS/MS profiles obtained with a standard mixture of 19 phenolic acids and their derivatives (100 pmol each). Separations were performed on a Waters HSS T3 C18 column (1 x 100 mm, 1.8 μ m particle size) using water as eluent A and 90% aq. CH_3CN as eluent B both containing 0.01% $\text{NH}_4\text{CH}_3\text{COO}$ with pH 5.0 (Panel A), pH 4.5 (Panel B), pH 4.0 (Panel C). The peaks are marked as in Table 1.

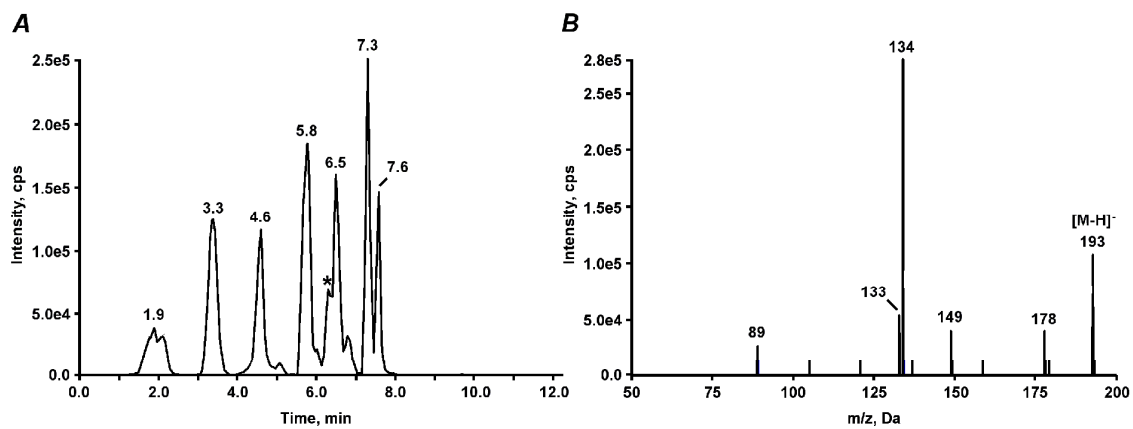


Fig S3 TIC-chromatogram (with analyte t_R marked) obtained in the survey MRM scan of an IDA experiment performed with a standard mixture of 19 phenolics (10 pmol each, Panel A) and MS/MS spectrum of m/z 193.1 (corresponding to ferulic acid) acquired in the dependent EPI scan at 6.3 min (asterisk) of the same IDA experiment (Panel B).

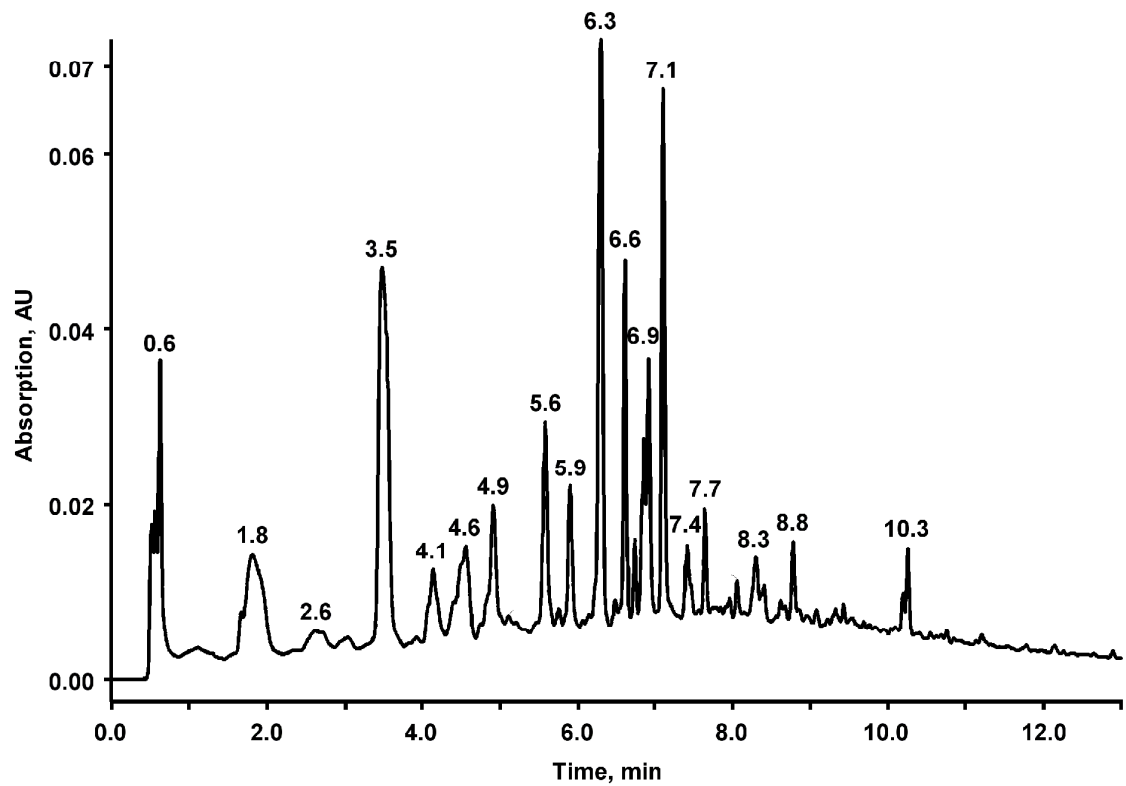


Fig S4 Reversed phase UV-chromatogram of saponified cell wall material obtained from *B. napus* seeds. Detection was performed by absorption at 280 nm.

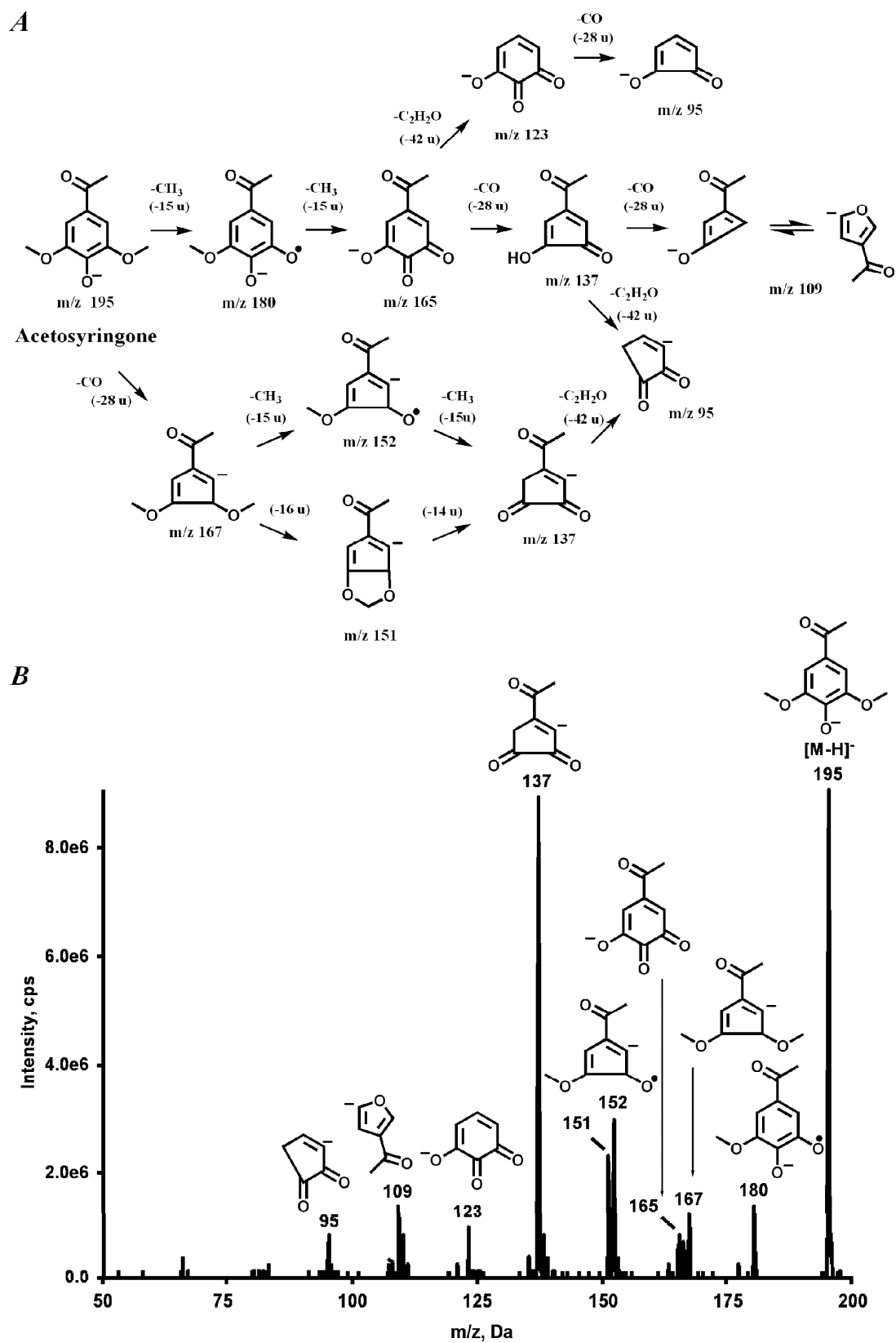


Fig S5 Proposed fragmentation pattern of acetosyringone (Panel A) and matching of this pattern to the MS/MS spectrum acquired at 7.9 min of IDA experiment presented in the Figure 3 (Panel B).