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Evaluation and application of a targeted SPE-LC-MS method for quantifying plant hormones and phenolics in Arabidopsis

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Supplementary material

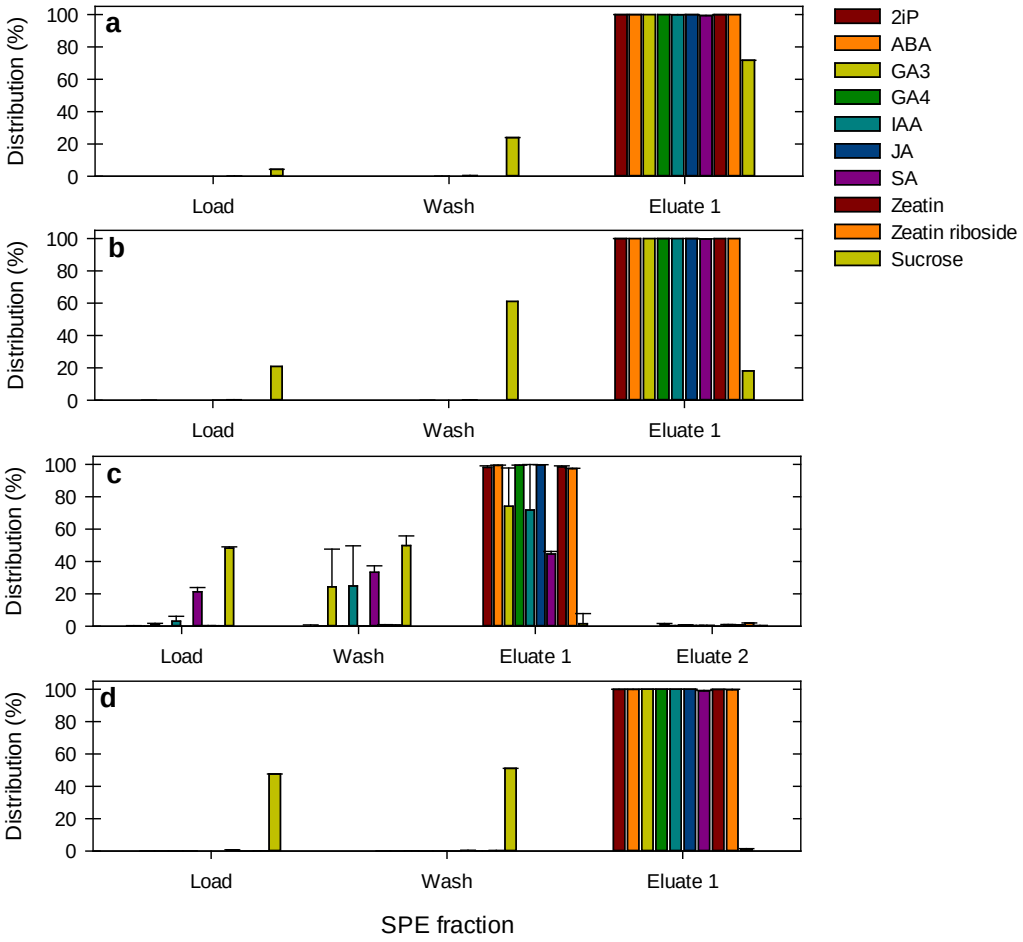

Fig. S1. Comparison of the performance of solid phase extraction (SPE) columns with selected compounds. The distribution (in %) of the total amount collected is shown for each compound, using four types of SPE columns: 2-pyrrolidinone grafted reversed phase (Oasis HLB, Waters, **a**), sulfonated reversed strong cation exchange phase (Oasis MCX, Waters, or Strata-X-C, Phenomenex) (**b**), C_{18} reversed phase (Strata C18, Phenomenex, **c**) and 1,2-ethanediamine grafted weak anion exchange phase (Strata X-AW, Phenomenex, **d**). In this trial, LC-MS analysis has been done in negative ionization only. Data shown are mean±SE (n = 3).

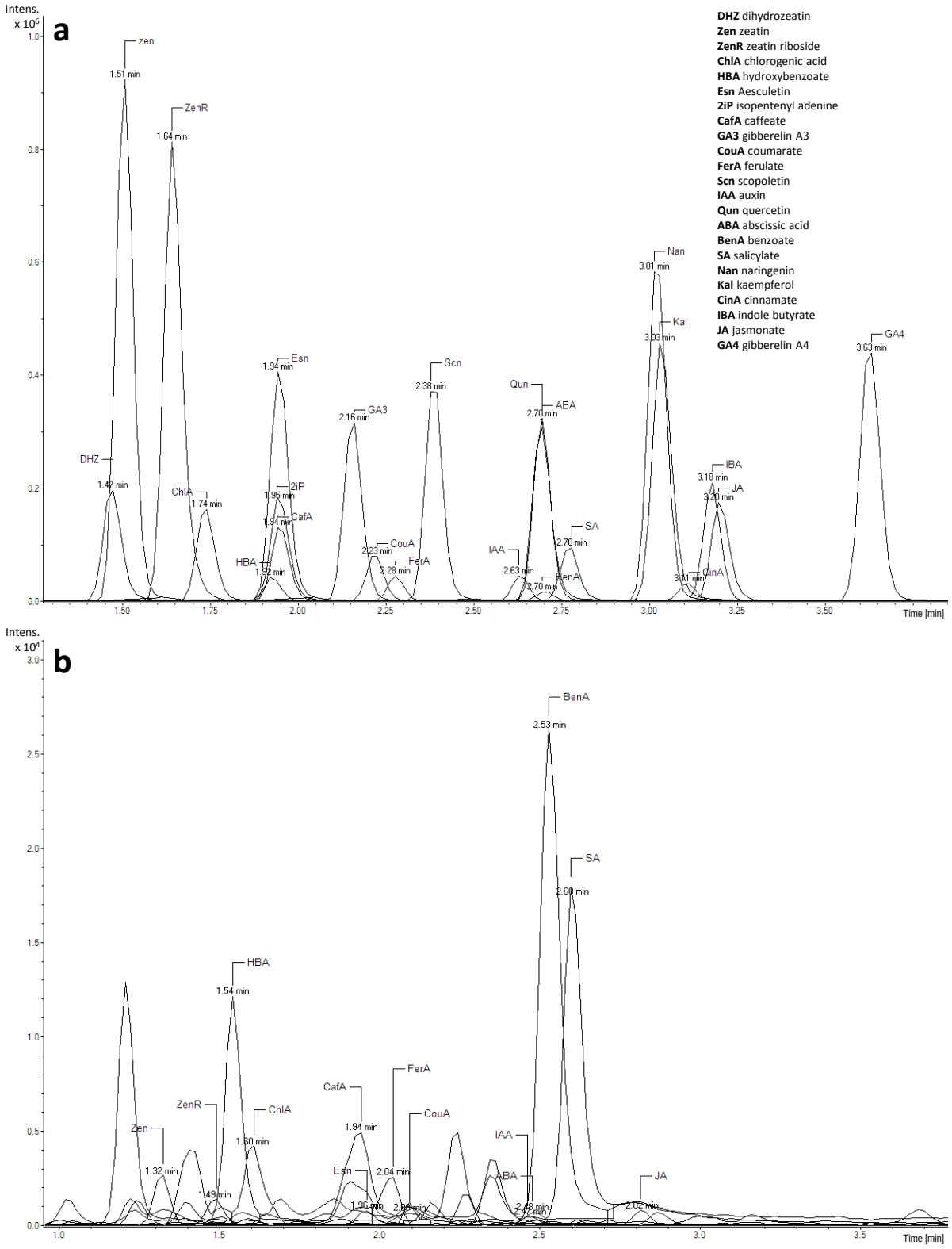

Fig. S2. Typical extracted ion chromatograms (monoisotopic parental ions) in negative ionization, showing the compounds of interest in a standard mixture (**a**) or in a sample from Arabidopsis leaf (**b**). Abbreviations are recalled on top.

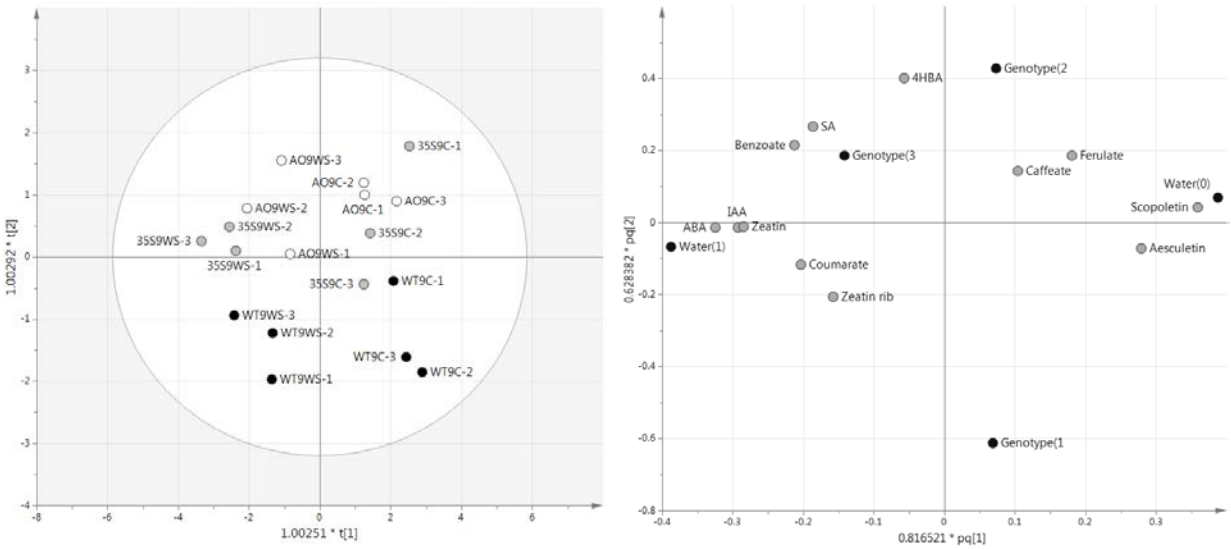

Fig. S3. Ouput of the multivariate OPLS analysis, showing the scatter plot (left) and the loading plot (right). In the scatter plot, samples are discriminated along two axes: control vs. water stress (axis-1) and WT vs. mutant lines (axis-2). Control samples are in black, *ao* in white and 35S in gray. Samples are labelled with the name, time (0 and 9 days), condition (C, control; WS, water stress) and the no. of replicate. The loading plot shows that the most visible changes are an enrichment in abscisic acid and a depletion in scopoletin upon water stress, and an enrichment in 4-hydroxybenzoate in the mutant *ao*. The OPLS is associated with a correlation coefficient R^2 between observed and modelled distribution of 0.78, and a cross-validated coefficient Q^2 of 0.49. The OPLS model is associated with a significant effect of water stress ($P = 0.004$) while the genotype effect is found to be insignificant ($P = 0.77$), as indicated by the ANOVA of the regression.

Table S1 (next page). List of targeted ions including adducts. N and P stand for negative and positive ionization, respectively.

Metabolite	Abbreviation	Ion species	Ion type	Ionisation mode	Ion name	Formula	Charge	m/z	Pubchem link
Isopentenyl adenine	2IP	Parental	[M-H]-	N	2IP	C10H12N5^-1	-1	202.108712	https://pubchem.ncbi.nlm.nih.gov/compound/Isopentenyladenine#section=Top
Isopentenyl adenine	2IP	Chloride adduct	[M+Cl]-	N	2IP_Cl	C10H13N5Cl^-1	-1	238.085400	
Abcissic acid	ABA	Parental	[M-H]-	N	ABA	C15H19O4^-1	-1	263.127786	https://pubchem.ncbi.nlm.nih.gov/compound/5280896#section=Top
Abcissic acid	ABA	Dimer	[M-H]-	N	2ABA	C30H39O8^-1	-1	527.263945	
Abcissic acid	ABA	Sodium adduct of dimer	[M-2H+Na]-	N	2ABA_Na	C30H38NaO8^-1	-1	549.245889	
Benzoate	BenA	Parental	[M-H]-	N	Benzoic_Acid	C7H5O2^-1	-1	121.028406	https://pubchem.ncbi.nlm.nih.gov/compound/243
Caffeate	CafA	Parental	[M-H]-	N	Caffeic_Acid	C9H7O4^-1	-1	179.033850	https://pubchem.ncbi.nlm.nih.gov/compound/689043
Caffeate	CafA	Decarboxylated	[M-H]-	N	Caffeic_Acid-COOH	C8H7O2^-1	-1	135.045153	
Chlorogenate	ChlA	Parental	[M-H]-	N	Chlorogenic_Acid	C16H17O9^-1	-1	353.086709	https://pubchem.ncbi.nlm.nih.gov/compound/1794427#section=Top
Chlorogenate	ChlA	Fragment (quinic moity)	[M-H]-	N	Chlorogenic_Acid_2	C7H11O6^-1	-1	191.055015	
Chlorogenate	ChlA	Sodium adduct	[M-2H+Na]-	N	Chlorogenic_Acid_Na	C16H16NaO9^-1	-1	375.068653	
Cinnamate	CinA	Parental	[M-H]-	N	Cinnamic_Acid	C9H7O2^-1	-1	147.044560	https://pubchem.ncbi.nlm.nih.gov/compound/444539
Coumarate	CouA	Parental	[M-H]-	N	Coumaric_Acid	C9H7O3^-1	-1	163.038971	https://pubchem.ncbi.nlm.nih.gov/compound/637542
Coumarate	CouA	Decarboxylated	[M-H]-	N	Coumaric_Acid-COOH	C8H7O^-1	-1	119.050238	
Dihydrozeatin	DHZ	Parental	[M-H]-	N	Dihydrozeatin	C10H14N5O^-1	-1	220.119287	https://pubchem.ncbi.nlm.nih.gov/compound/439631
Dihydrozeatin	DHZ	Chloride adduct	[M+Cl]-	N	Dihydrozeatin_Cl	C10H15N5ClO^-1	-1	256.095640	
Aesculetin	Esn	Parental	[M-H]-	N	Esculetin	C9H5O4^-1	-1	177.018235	https://pubchem.ncbi.nlm.nih.gov/compound/5281416
Ferulate	FerA	Parental	[M-H]-	N	Ferulic_Acid	C10H9O4^-1	-1	193.049535	https://pubchem.ncbi.nlm.nih.gov/compound/445858
Ferulate	FerA	Fragment (hydroxymethoxybenzyl moiety)	[M-H]-	N	Ferulic_Acid-CHCOOH	C8H7O2^-1	-1	134.036231	
Ferulate	FerA	Demethylated	[M-H]-	N	Ferulic_Acid-CH3	C9H6O4^-1	-1	178.026060	
Gibberelin A3	GA3	Parental	[M-H]-	N	GA3	C19H21O6^-1	-1	345.133265	https://pubchem.ncbi.nlm.nih.gov/compound/439551
Gibberelin A3	GA3	Dimer	[M-H]-	N	2GA3	C38H43O12^-1	-1	691.274903	
Gibberelin A4	GA4	Parental	[M-H]-	N	GA4	C19H23O5^-1	-1	331.154000	https://pubchem.ncbi.nlm.nih.gov/compound/92109
Gibberelin A4	GA4	Dimer	[M-H]-	N	2GA4	C38H47O10^-1	-1	663.316374	
4-Hydroxybenzoate	HBA	Parental	[M-H]-	N	Hydroxybenzoic_Acid	C7H5O3^-1	-1	137.023320	https://pubchem.ncbi.nlm.nih.gov/compound/135
4-Hydroxybenzoate	HBA	Decarboxylated	[M-H]-	N	Hydroxybenzoic_Acid-COOH	C6H5O^-1	-1	93.034588	
Auxin	IAA	Parental	[M-H]-	N	IAA	C10H8NO2^-1	-1	174.056052	https://pubchem.ncbi.nlm.nih.gov/compound/802#section=Top
Auxin	IAA	Decarboxylated	[M-H]-	N	IAA-COOH	C9H8N^-1	-1	130.066223	
3-indole byturate	IBA	Parental	[M-H]-	N	IBA	C12H12NO2^-1	-1	202.087352	https://pubchem.ncbi.nlm.nih.gov/compound/8617
3-indole byturate	IBA	Sodium adduct	[M-2H+Na]-	N	IBA_Na	C12H11NNaO2^-1	-1	224.069297	
3-indole byturate	IBA	Sodium adduct of dimer	[M-2H+Na]-	N	2IBA_Na	C24H24N2NaO4^-1	-1	427.163926	
Jasmonate	JA	Parental	[M-H]-	N	JA	C12H17O3^-1	-1	209.118318	https://pubchem.ncbi.nlm.nih.gov/compound/5281166
Jasmonate	JA	Sodium adduct of dimer	[M-2H+Na]-	N	2JA_Na	C24H34NaO6^-1	-1	441.225857	
Kaempferol	Kal	Parental	[M-H]-	N	Kaempferol	C15H9O6^-1	-1	285.040462	https://pubchem.ncbi.nlm.nih.gov/compound/5280863
Naringenin	Nan	Parental	[M-H]-	N	Naringenin	C15H11O5^-1	-1	271.061197	https://pubchem.ncbi.nlm.nih.gov/compound/932
Naringenin	Nan	Dimer	[M-H]-	N	2Naringenin	C30H23O10^-1	-1	543.129471	
Quercetin	Qun	Parental	[M-H]-	N	Quercetin	C15H9O7^-1	-1	301.035376	https://pubchem.ncbi.nlm.nih.gov/compound/932
Salicylate	SA	Parental	[M-H]-	N	SA	C7H5O3^-1	-1	137.024418	https://pubchem.ncbi.nlm.nih.gov/compound/338
Salicylate	SA	Decarboxylated	[M-H]-	N	SA-COOH	C6H5O^-1	-1	93.034588	
Scopoletin	Scn	Parental	[M-H]-	N	Scopoletin	C10H7O4^-1	-1	191.034982	https://pubchem.ncbi.nlm.nih.gov/compound/5280460
Scopoletin	Scn	Demethylated	[M-H]-	N	Scopoletin-CH3	C9H4O4^-1	-1	176.011507	
Zeatin	Zen	Parental	[M-H]-	N	Z	C10H12N5O^-1	-1	218.104734	https://pubchem.ncbi.nlm.nih.gov/compound/449093
Zeatin	Zen	Chloride adduct	[M+Cl]-	N	Z_Cl	C10H13ClN5O^1-	-1	254.081411	
Zeatin	Zen	Dehydrated	[M-H]-	N	Z-H2O	C10H10N5^-1	-1	200.094169	
Zeatin riboside	ZenR	Carboxylated adduct	[M-H]-	N	Z_Riboside+COOH	C16H21N5O7^-1	-1	396.151375	
Zeatin riboside	ZenR	Parental	[M-H]-	N	Z_Riboside	C15H20N5O5^-1	-1	350.146992	https://pubchem.ncbi.nlm.nih.gov/compound/6440982#section=Top
Zeatin riboside	ZenR	Chloride adduct	[M+Cl]-	N	Z_Riboside_Cl	C15H21ClN5O5^-1	-1	386.123670	
Zeatin riboside	ZenR	Fragment (hydroxymethylbutenyl adenine)	[M-H]-	N	Z_Riboside-ribose	C10H12N5O^-1	-1	218.104734	
Isopentenyl adenine	2IP	Parental	[M+H]+	P	2iP	C10H14N5^+1	+1	204.124372	https://pubchem.ncbi.nlm.nih.gov/compound/Isopentenyladenine#section=Top
Isopentenyl adenine	2IP	Fragment (adenine moiety)	[M+H]+	P	2iP_2	C5H5N5^+1	+1	136.061772	
Isopentenyl adenine	2IP	Fragment (methenyl adenine)	[M+H]+	P	2iP_3	C6H6N5^+1	+1	148.061772	
Isopentenyl adenine	2IP	Fragment (deamino adenine)	[M+H]+	P	2iP_4	C5H3N4^+1	+1	119.035223	
Abscissic acid	ABA	Parental	[M+H]+	P	ABA	C15H21O4^+1	+1	265.143436	https://pubchem.ncbi.nlm.nih.gov/compound/5280896#section=Top
Abscissic acid	ABA	Bis-dehydrated	[M+H]+	P	ABA_2	C15H17O2^+1	+1	229.122306	
Abscissic acid	ABA	Decarboxylated	[M+H]+	P	ABA_3	C14H19O2^+1	+1	219.137956	
Abscissic acid	ABA	Decarboxylated and dehydrated	[M+H]+	P	ABA_4	C14H17O^+1	+1	201.127392	
Abscissic acid	ABA	Decarboxylated and demethylated	[M+H]+	P	ABA_5	C13H17O2^+1	+1	205.122306	
Abscissic acid	ABA	Decarboxylated and demethylated and dehydrated	[M+H]+	P	ABA_6	C13H15O^+1	+1	187.111742	
Abscissic acid	ABA	Potassium adduct	[M+K]+	P	ABA_K	C15H20KO4^+1	+1	303.099317	
Abscissic acid	ABA	Sodium adduct	[M+Na]+	P	ABA_Na	C15H20NaO4^+1	+1	287.125380	
Abscissic acid	ABA	Ammonium adduct	[M+NH4]+	P	ABA_NH4	C15H24NO4^+1	+1	282.169985	

Abscissic acid	ABA	Dehydrated	[M+H]+	P	ABA-H2O	C15H19O3^+1	+1	247.132871	
Abscissic acid	ABA	Dimer	[M+H]+	P	2ABA	C30H41O8^+1	+1	529.279595	
Abscissic acid	ABA	Sodium adduct of dimer	[M+Na]+	P	2ABA_Na	C30H40NaO8^+1	+1	551.261539	
Benzoate	BenA	Parental	[M+H]+	P	Benzoic_Acid	C7H7O2^+1	+1	123.044056	https://pubchem.ncbi.nlm.nih.gov/compound/243
Caffeate	CafA	Parental	[M+H]+	P	Caffeic_Acid	C9H9O4^+1	+1	181.049535	https://pubchem.ncbi.nlm.nih.gov/compound/689043
Caffeate	CafA	Dehydrated	[M+H]+	P	Caffeic_Acid-H2O	C9H7O3^+1	+1	163.038971	
Chlorogenate	ChlA	Parental	[M+H]+	P	Chlorogenic_Acid	C16H19O9^+1	+1	355.102359	https://pubchem.ncbi.nlm.nih.gov/compound/1794427#section=Top
Chlorogenate	ChlA	Fragment (caffeate moiety)	[M+H]+	P	Chlorogenic_Acid_2	C9H7O3^+1	+1	163.038971	
Chlorogenate	ChlA	Sodium adduct	[M+Na]+	P	Chlorogenic_Acid_Na	C16H18NaO9^+1	+1	377.084303	
Cinnamate	CinA	Parental	[M+H]+	P	Cinnamic_Acid	C9H9O2^+1	+1	149.059706	https://pubchem.ncbi.nlm.nih.gov/compound/444539
Cinnamate	CinA	Decarboxylated and deprotonated	[M+H]+	P	Cinnamic_Acid-COOH	C8H7^+1	+1	103.054227	
Cinnamate	CinA	Dehydrated	[M+H]+	P	Cinnamic_Acid-H2O	C9H7O^+1	+1	131.049141	
Coumarate	CouA	Parental	[M+H]+	P	Coumaric_Acid	C9H9O3^+1	+1	165.054621	https://pubchem.ncbi.nlm.nih.gov/compound/637542
Coumarate	CouA	Decarboxylated and deprotonated	[M+H]+	P	Coumaric_Acid-COOH	C8H7O^+1	+1	119.049141	
Coumarate	CouA	Dehydrated	[M+H]+	P	Coumaric_Acid-H2O	C9H7O2^+1	+1	147.044056	
Dihydrozeatin	DHZ	Parental	[M+H]+	P	Dihydrozeatin	C10H16N5O^+1	+1	222.134937	https://pubchem.ncbi.nlm.nih.gov/compound/439631
Dihydrozeatin	DHZ	Fragment (adenine moiety)	[M+H]+	P	Dihydrozeatin_2	C5H6N5^+1	+1	136.061772	
Aesculetin	Esn	Parental	[M+H]+	P	Esculetin	C9H7O4^+1	+1	179.033885	https://pubchem.ncbi.nlm.nih.gov/compound/5281416
Ferulate	FerA	Parental	[M+H]+	P	Ferulic_Acid	C10H11O4^+1	+1	195.065185	https://pubchem.ncbi.nlm.nih.gov/compound/445858
Ferulate	FerA	Demethoxylated and dehydroxylated and oxidised	[M+H]+	P	Ferulic_Acid_2	C9H5O2^+1	+1	145.028406	
Ferulate	FerA	Dehydrated	[M+H]+	P	Ferulic_Acid-H2O	C10H9O3^+1	+1	177.054621	
Gibberelin A3	GA3	Parental	[M+H]+	P	GA3	C19H23O6+1	+1	347.148915	https://pubchem.ncbi.nlm.nih.gov/compound/439551
Gibberelin A3	GA3	Bis-dehydrated	[M+H]+	P	GA3_2	C19H19O4^+1	+1	311.127786	
Gibberelin A3	GA3	Decarboxylated and dehydroxylated	[M+H]+	P	GA3_3	C18H21O3^+1	+1	285.148521	
Gibberelin A3	GA3	Decarboxylated and dehydrated and oxidised	[M+H]+	P	GA3_4	C18H19O3^+1	+1	283.132871	
Gibberelin A3	GA3	Decarboxylated and dehydrated and dehydroxylated	[M+H]+	P	GA3_5	C18H19O2^+1	+1	267.137956	
Gibberelin A3	GA3	Dehydrated	[M+H]+	P	GA3-H2O	C19H21O5^+1	+1	329.138350	
Gibberelin A4	GA4	Parental	[M+H]+	P	GA4	C19H25O5^+1	+1	333.169650	https://pubchem.ncbi.nlm.nih.gov/compound/92109
Gibberelin A4	GA4	Decarboxylated and dehydroxylated	[M+H]+	P	GA4_2	C18H23O3^+1	+1	287.164171	
Gibberelin A4	GA4	Decarboxylated and dehydrated and oxidised	[M+H]+	P	GA4_3	C18H21O2^+1	+1	269.153606	
Gibberelin A4	GA4	Bis-decarboxylated and dehydrated	[M+H]+	P	GA4_4	C17H21^+1	+1	225.163777	
Gibberelin A4	GA4	Sodium adduct	[M+Na]+	P	GA4_Na	C19H24NaO5^+1	+1	355.151595	
Gibberelin A4	GA4	Dehydrated	[M+H]+	P	GA4-H2O	C19H23O4^+1	+1	315.159086	
4-Hydroxybenzoate	HBA	Parental	[M+H]+	P	Hydroxybenzoic_Acid	C7H7O3+1	+1	139.038971	https://pubchem.ncbi.nlm.nih.gov/compound/135
4-Hydroxybenzoate	HBA	Dehydrated	[M+H]+	P	Hydroxybenzoic_Acid-H2O	C7H5O2^+1	+1	121.028406	
Auxin	IAA	Parental	[M+H]+	P	IAA	C10H10NO2^+1	+1	176.070605	https://pubchem.ncbi.nlm.nih.gov/compound/802#section=Top
Auxin	IAA	Decarboxylated and oxidised	[M+H]+	P	IAA-COOH	C9H8N^+1	+1	130.065126	
3-indole byturate	IBA	Parental	[M+H]+	P	IBA	C12H14NO2^+1	+1	204.101905	https://pubchem.ncbi.nlm.nih.gov/compound/8617
3-indole byturate	IBA	Dehydrated	[M+H]+	P	IBA-H2O	C12H12NO^+1	+1	186.091340	
Jasmonate	JA	Parental	[M+H]+	P	JA	C12H19O3^+1	+1	211.132871	https://pubchem.ncbi.nlm.nih.gov/compound/5281166
Jasmonate	JA	Deacetylated	[M+H]+	P	JA_2	C10H15O^+1	+1	151.111742	
Jasmonate	JA	Deacetylated and dehydrated	[M+H]+	P	JA_3	C10H13^+1	+1	133.101177	
Jasmonate	JA	Sodium adduct	[M+Na]+	P	JA_Na	C12H18NaO3^+1	+1	233.114816	
Jasmonate	JA	Dehydrated	[M+H]+	P	JA-H2O	C12H17O2^+1	+1	193.122306	
Kaempferol	Kal	Parental	[M+H]+	P	Kaempferol	C15H11O6^+1	+1	287.055015	https://pubchem.ncbi.nlm.nih.gov/compound/5280863
Naringenin	Nan	Parental	[M+H]+	P	Naringenin	C15H13O5^+1	+1	273.075750	https://pubchem.ncbi.nlm.nih.gov/compound/932
Quercetin	Qun	Parental	[M+H]+	P	Quercetin	C15H11O7^+1	+1	303.049929	https://pubchem.ncbi.nlm.nih.gov/compound/932
Salicylate	SA	Parental	[M+H]+	P	SA	C7H7O3^+1	+1	139.038971	https://pubchem.ncbi.nlm.nih.gov/compound/338
Salicylate	SA	Dehydrated	[M+H]+	P	SA-H2O	C7H5O2^+1	+1	121.028406	
Scopoletin	Scn	Parental	[M+H]+	P	Scopoletin	C10H9O4^+1	+1	193.049535	https://pubchem.ncbi.nlm.nih.gov/compound/5280460
Scopoletin	Scn	Sodium adduct	[M+Na]+	P	Scopoletin_Na	C10H8NaO4^+1	+1	215.031480	
Zeatin	Zen	Parental	[M+H]+	P	Z	C10H14N5O^+1	+1	220.119287	https://pubchem.ncbi.nlm.nih.gov/compound/449093
Zeatin	Zen	Fragment (adenine moiety)	[M+H]+	P	Z_2	C5H6N5^+1	+1	136.066177	
Zeatin	Zen	Fragment (methenyl adenine)	[M+H]+	P	Z_3	C6H6N5^1+	+1	148.061772	
Zeatin	Zen	Dehydrated	[M+H]+	P	Z-H2O	C10H12N5^+1	+1	202.108722	
Zeatin riboside	ZenR	Parental	[M+H]+	P	Z_Riboside	C15H22N5O5^+1	+1	352.161545	https://pubchem.ncbi.nlm.nih.gov/compound/6440982#section=Top
Zeatin riboside	ZenR	Fragment (zeatin moiety)	[M+H]+	P	Z_Riboside-ribose	C10H14N5O^+1	+1	220.119287	