# Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

# In

# Metabolomics

## FT-ICR MS or FTMS

# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

- Will determain the m/z ratio of an ion

- Ions are formed as in other mass analysers: e.g. Electrospray Ionization (ESI); Matrix Assisted laser Desorption Ionization (MALDI); Electron Ionization (EI); Chemical Ionization (CI)

# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

- The m/z ratio measurment of an ion is based upon the ion's motion or cyclotron frequency in a magnetic field

- Ions are detected by passing near detection plates and thus differently from other mass detectors/analysers in which ions are hitting a detector (at diferent times or places)

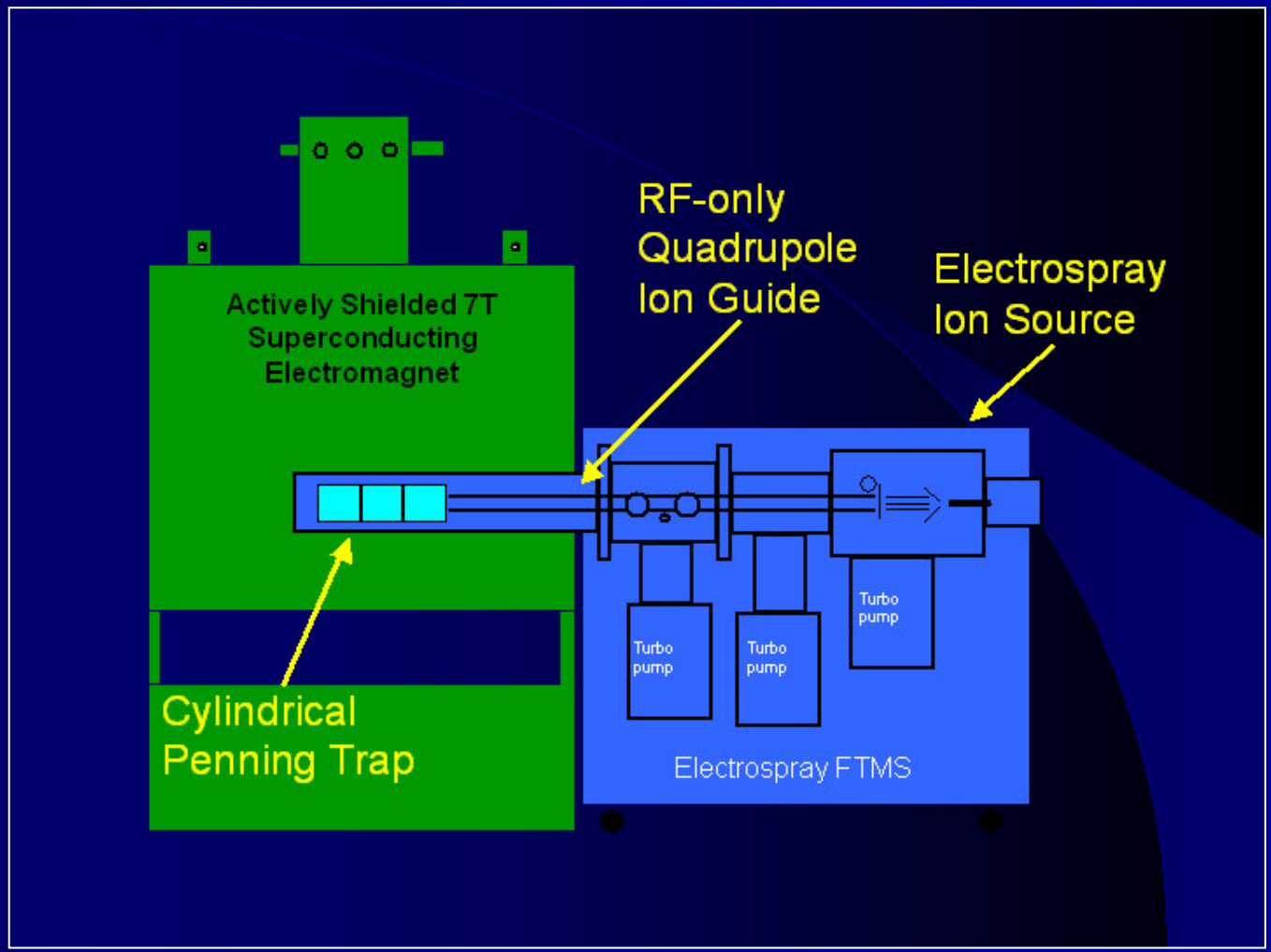
# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

- The ions are trapped in a magnetic field combined with electric field perpendicular to each other (Penning trap)

- They are excited to perform a cyclotron motion

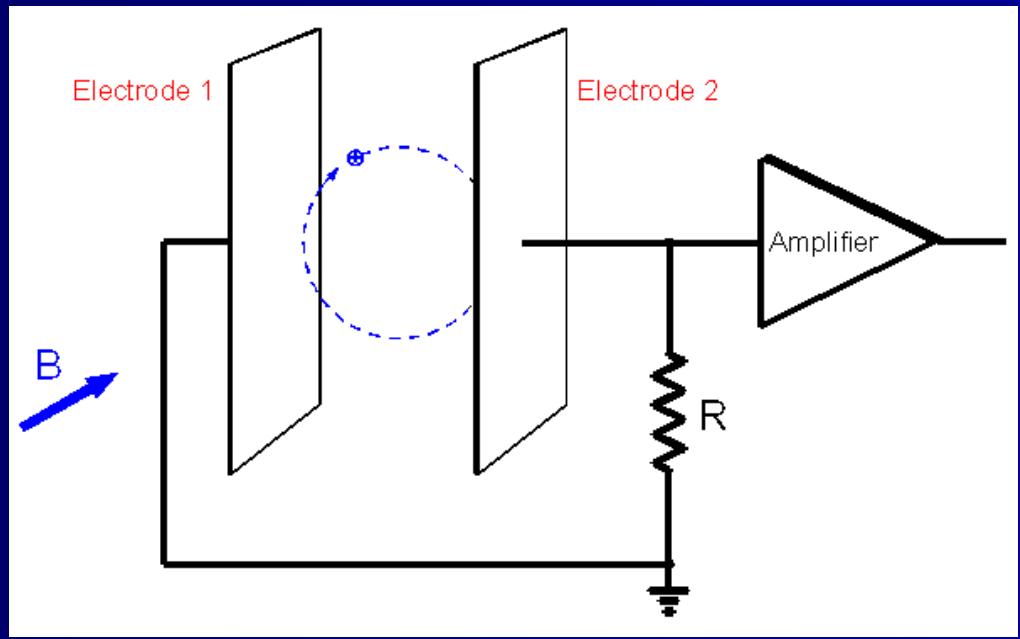
- The cyclotron frequency depends on the ratio of electric charge to mass (m/z) and strength of the magnetic field

# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry



# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

- This frequency signal "image" (Ion Cyclotron Resonance, ICR signal) is detected by a pair of opposing electrode plates



# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

- The ICR signal is converted to a frequency spectrum by Fourier transformation

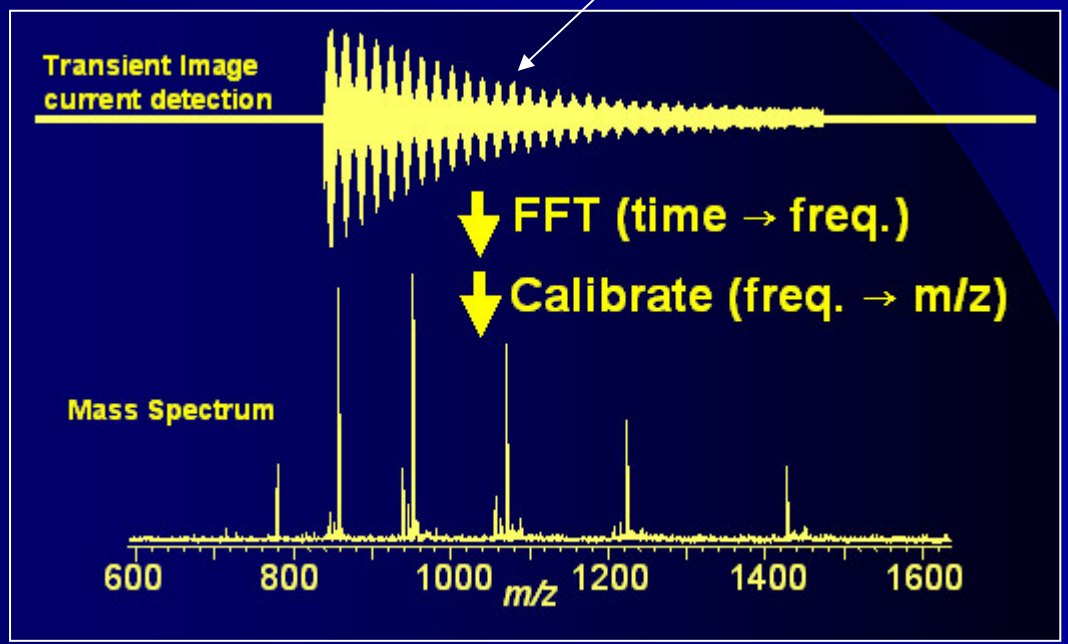
- The relation between the cyclotron frequency and the m/z could be simply presented as:

$$f = 15357 \cdot Bz/m$$

f = ion cyclotron frequency in kHz
B = is the magnetic field strength in Tesla
z = is the charge state of the ion
M = is the mass of the ion in Da.

# Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

The frequency spectrum is converted to a mass spectrum



# FTMS Characteristcs

- The highest resolving power of all mass analyzers

- The highest mass accuracy- usually around 1ppm or less

**Ability of a mass analyzer to assign the mass of an ion close to its true value (exact mass)**

$\Delta m_{accuracy} = m_{real} - m_{measured}$

$\underline{\text{In ppm}} = 10^6 * \Delta m_{accuracy} / m_{measured}$

# FTMS Characteristcs

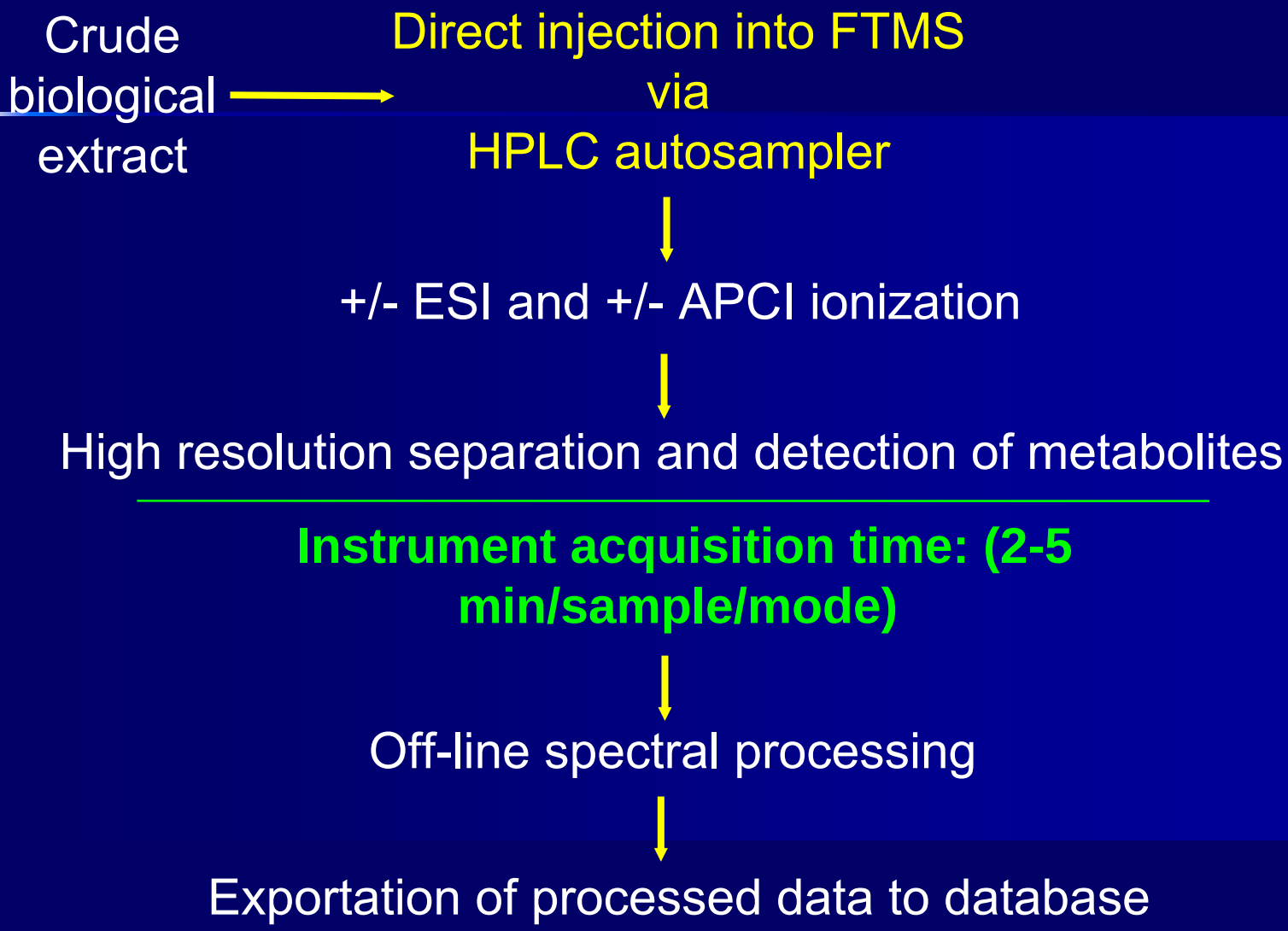
- Similar sensitivity to Q-TOF

- Limit of detection in MS/MS mode lower than for Q-TOF (Q-FTMS will be better)

- $MS^n$ could be performed

- Most ionization modes could be used

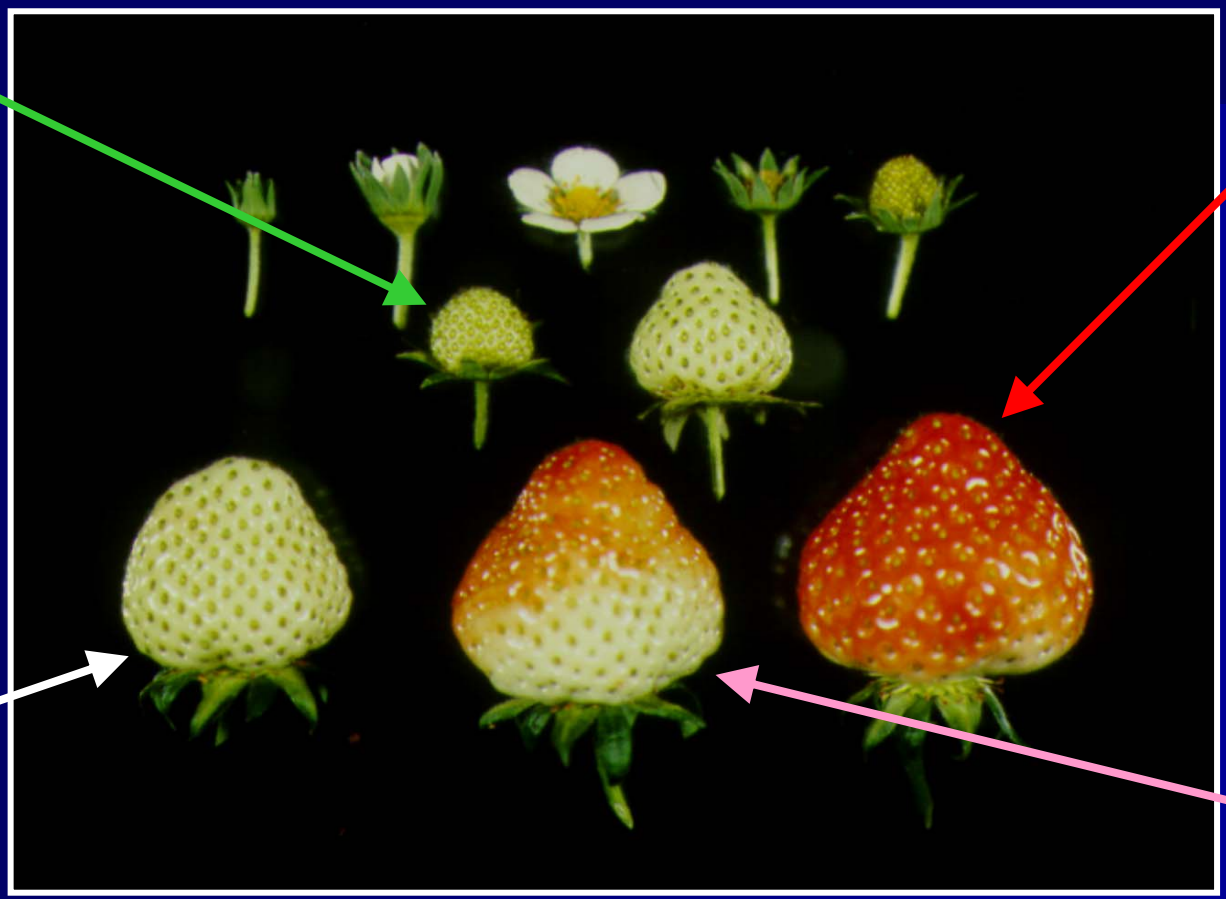
# FTMS metabolic phenotyping



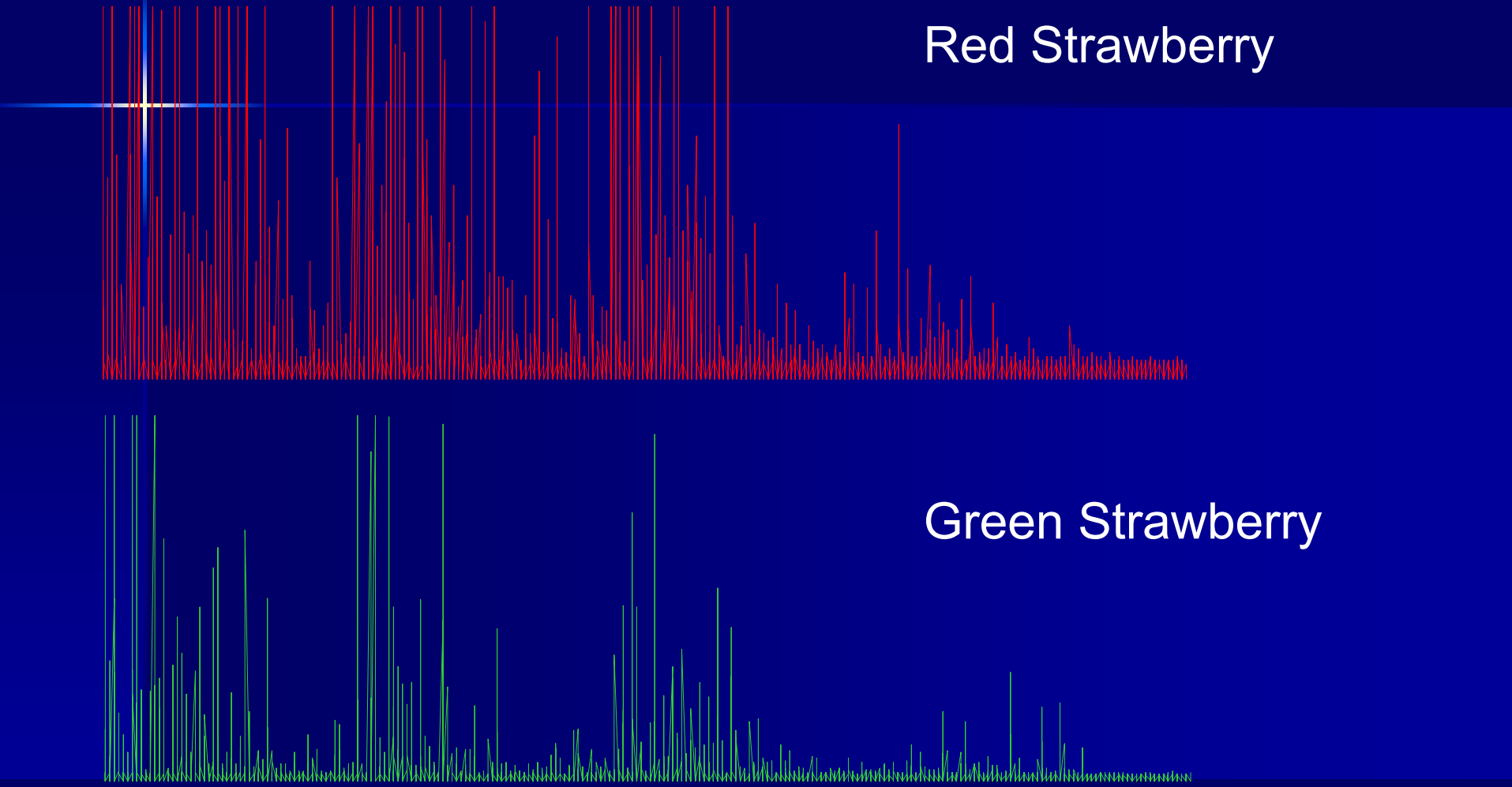
# Analytical Instrumentation (FTMS)


**Fourier Transform Ion Cyclotron Mass Spectrometry**

# Non-targeted metabolic profiling in strawberry

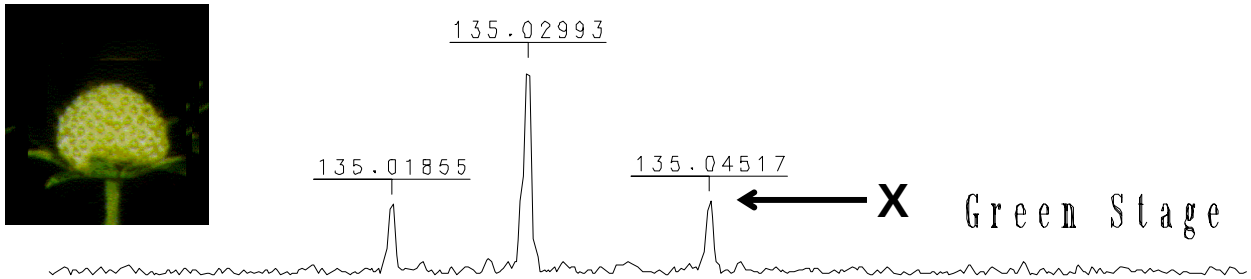


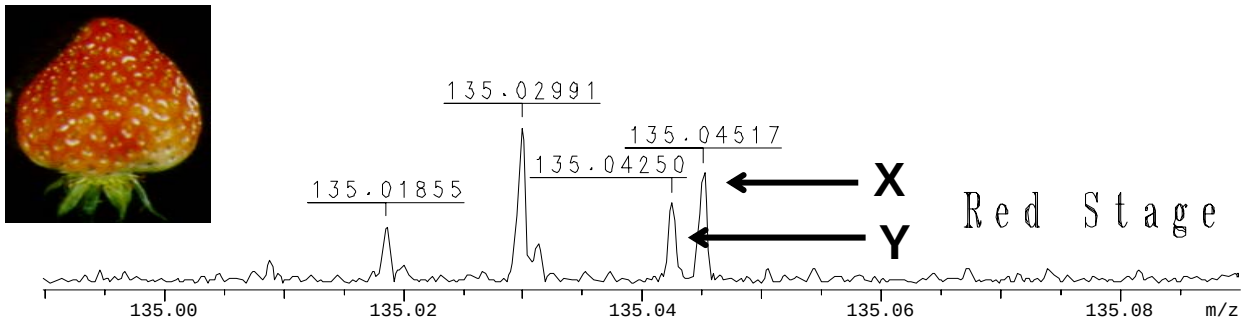
# Positive ion ESI of strawberry fruit



# High resolution separation

**X = 135.04517** **Y = 135.04250**




**Resolution = 135,000, 0.1 AMU Window**

# Empirical formula determination

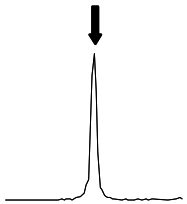
Internal Mass Calibration Of Sample

Calibration Results:

| Ref. Masses | Exp. Masses | Diff (ppm) |
|---|---|---|
| 124.039300 | 124.039309 | 0.0718 |
| 140.034220 | 140.034205 | 0.1064 |
| 303.166300 | 303.166457 | 0.5191 |
| 962.430130 | 962.430546 | 0.4319 |

Calculate Accurate Mass Of Metabolite

433.11270



Calculate Possible Empirical Formulas

Mass Analysis for mass 433.1127000

| # | 12C | 1H | 16O | 14N | 32S | mass | error |
|---|---|---|---|---|---|---|---|
| 1 | 21 | 21 | 10 | 0 | 0 | 433.1129233 | 5.156e-07 |
| 2 | 13 | 25 | 12 | 2 | 1 | 433.1122714 | 9.896e-07 |
| 3 | 11 | 13 | 2 | 16 | 1 | 433.1122608 | 1.014e-06 |
| 4 | 14 | 29 | 7 | 2 | 3 | 433.1131398 | 1.015e-06 |
| 5 | 11 | 21 | 1 | 12 | 3 | 433.1117918 | 2.097e-06 |
| 6 | 14 | 21 | 8 | 6 | 1 | 433.1136088 | 2.098e-06 |
| 7 | 26 | 17 | 1 | 4 | 1 | 433.1117583 | 2.174e-06 |
| 8 | 22 | 25 | 5 | 0 | 2 | 433.1137917 | 2.521e-06 |
| 9 | 18 | 13 | 4 | 10 | 0 | 433.1115754 | 2.597e-06 |
| 10 | 26 | 25 | 0 | 0 | 3 | 433.1112893 | 3.257e-06 |
| 11 | 22 | 17 | 6 | 4 | 0 | 433.1142607 | 3.603e-06 |
| 12 | 18 | 21 | 3 | 6 | 2 | 433.1111064 | 3.679e-06 |
| 13 | 10 | 17 | 6 | 12 | 1 | 433.1109234 | 4.102e-06 |
| 14 | 15 | 25 | 3 | 6 | 3 | 433.1144772 | 4.103e-06 |
| 15 | 15 | 17 | 4 | 10 | 1 | 433.1149462 | 5.186e-06 |
| 16 | 25 | 21 | 5 | 0 | 1 | 433.1104209 | 5.262e-06 |
| 17 | 23 | 21 | 1 | 4 | 2 | 433.1151291 | 5.609e-06 |
| 18 | 17 | 17 | 8 | 6 | 0 | 433.1102380 | 5.685e-06 |
| 19 | 23 | 13 | 2 | 8 | 0 | 433.1155981 | 6.691e-06 |
| 20 | 17 | 25 | 7 | 2 | 2 | 433.1097690 | 6.767e-06 |
| 21 | 10 | 29 | 12 | 2 | 2 | 433.1156422 | 6.793e-06 |
| 22 | 9 | 21 | 10 | 8 | 1 | 433.1095860 | 7.190e-06 |
| 23 | 10 | 21 | 13 | 6 | 0 | 433.1161112 | 7.876e-06 |
| 24 | 16 | 13 | 0 | 14 | 1 | 433.1162836 | 8.274e-06 |
| 25 | 17 | 19 | 5 | 7 | 1 | 433.1162889 | 8.286e-06 |
| 26 | 18 | 25 | 10 | 0 | 1 | 433.1162941 | 8.298e-06 |
| 27 | 16 | 21 | 12 | 2 | 0 | 433.1089006 | 8.772e-06 |

Assign Empirical Formula Based Upon Calibration Errors

$433.1127 = C_{21}H_{20}O_{10}[+H]^+$

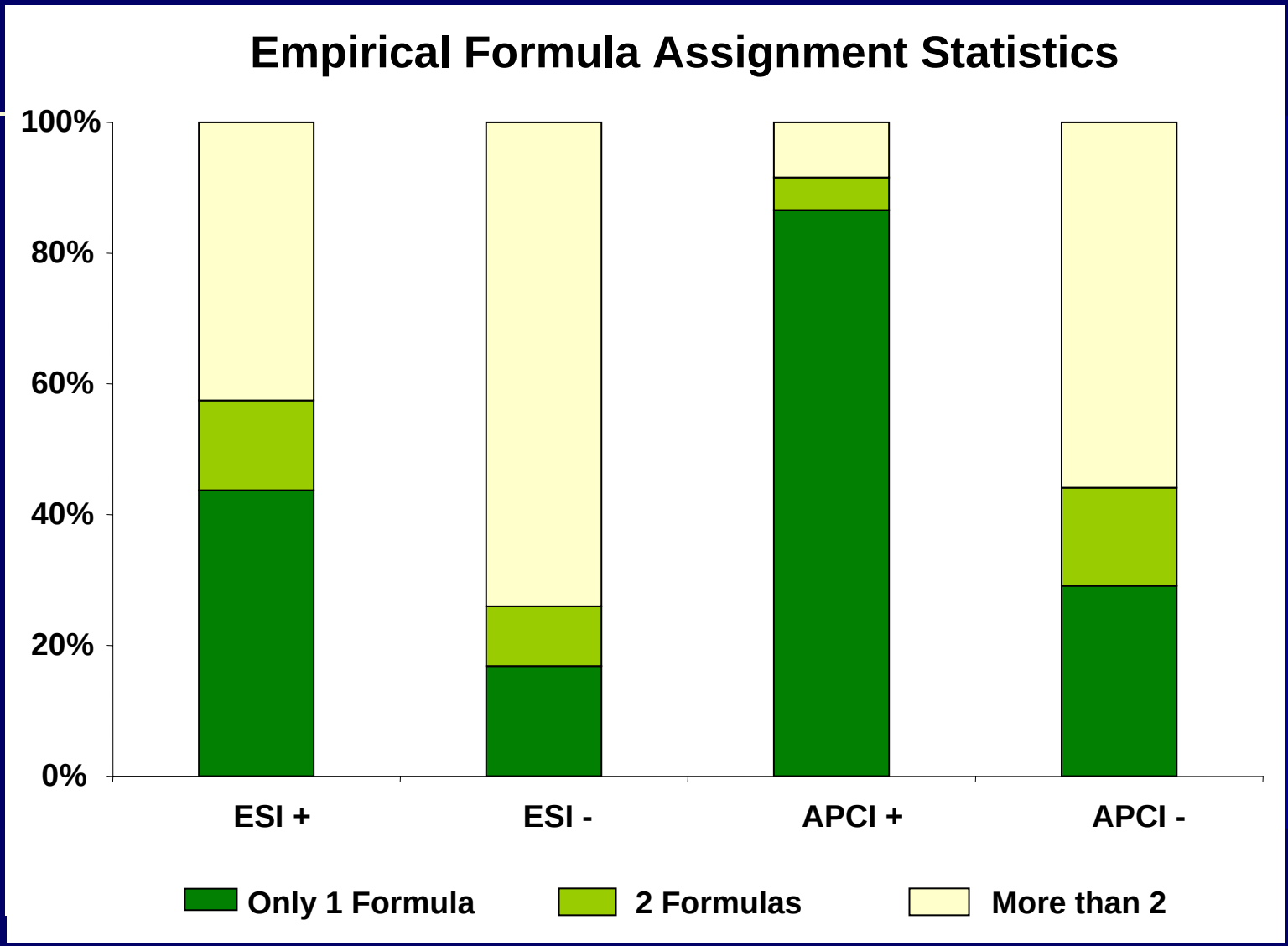
Error = 0.52ppm

# Number of mass peaks and metabolites observed

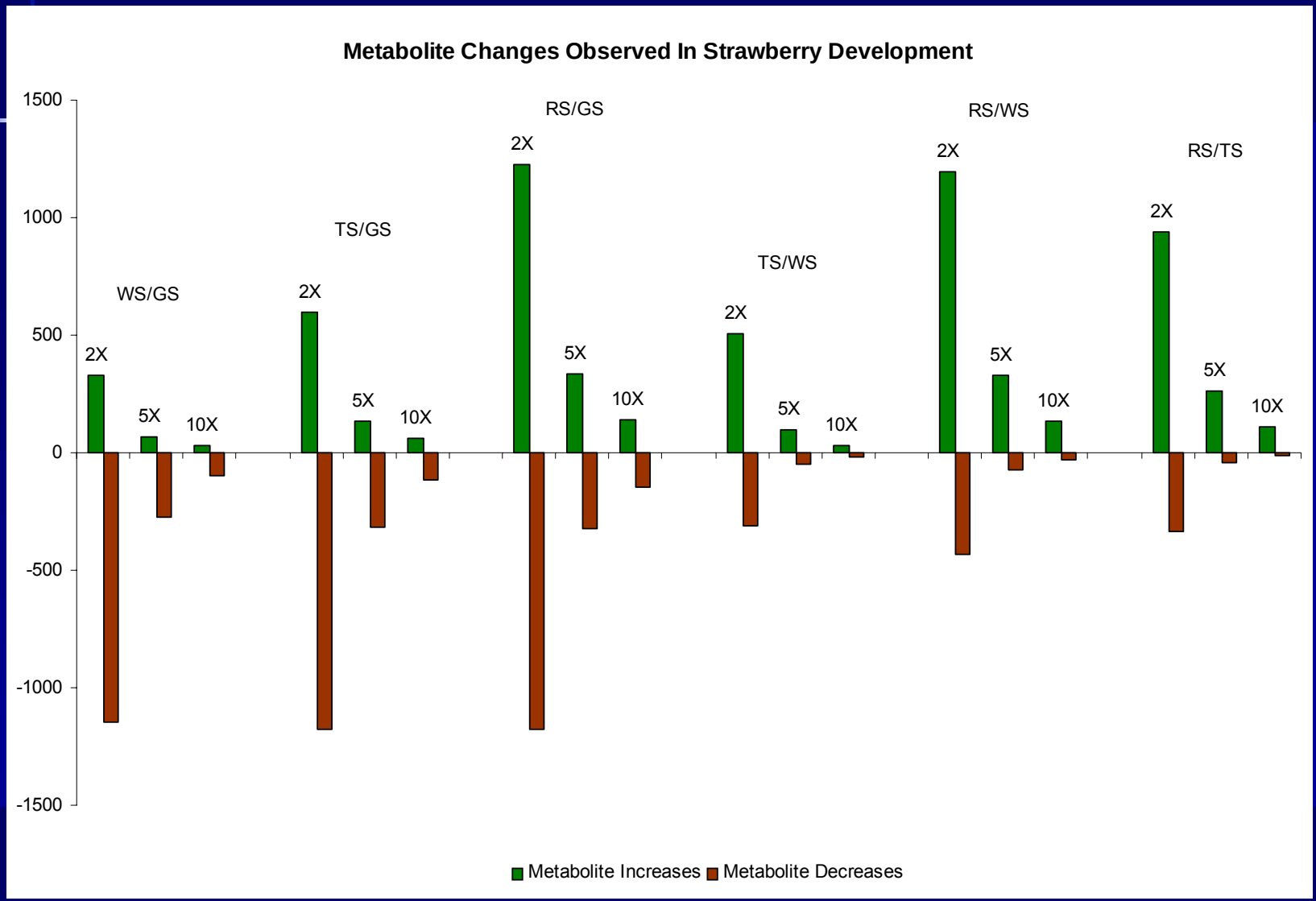
Number of metabolites observed across stages of fruit development, ionization modes and extractions

| | Overall mass peaks | | | | | | | | | Unique mass peaks | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Green | | White | | Turning | | Red | | Total | Only | | Both (%) | Total |
| | 50 | AN | 50 | AN | 50 | AN | 50 | AN | 50 + AN | 50 | AN | 50 + AN | |
| **ESI +** | 454 | 471 | 504 | 426 | 513 | 339 | 419 | 493 | 3619 | 578 | 520 | 374 (25%) | 1472 |
| **ESI-** | 513 | 285 | 315 | 247 | 269 | 319 | 348 | 193 | 2489 | 608 | 454 | 208 (16%) | 1270 |
| **APCI+** | 446 | 340 | 672 | 470 | 528 | 399 | 587 | 902 | 4344 | 368 | 595 | 536 (36%) | 1499 |
| **APCI-** | 519 | 546 | 211 | 435 | 260 | 296 | 233 | 460 | 2960 | 492 | 800 | 311 (19%) | 1603 |
| **Mean** | 483 | 411 | 426 | 395 | 393 | 338 | 397 | 512 | | 512 | 592 | 357 (24%) | 1461 |
| **Total** | 1932 | 1642 | 1702 | 1578 | 1570 | 1353 | 1587 | 2048 | **13,412** | 2046 | 2369 | 1429 (24%) | **5844** |

# Accurate empirical formula determination



# Quantitative determination of change between samples



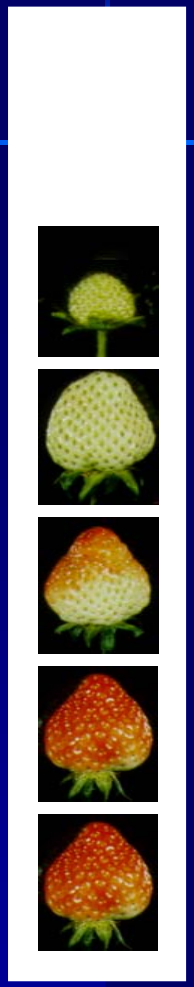
# Metabolic profiling of strawberry fruit development

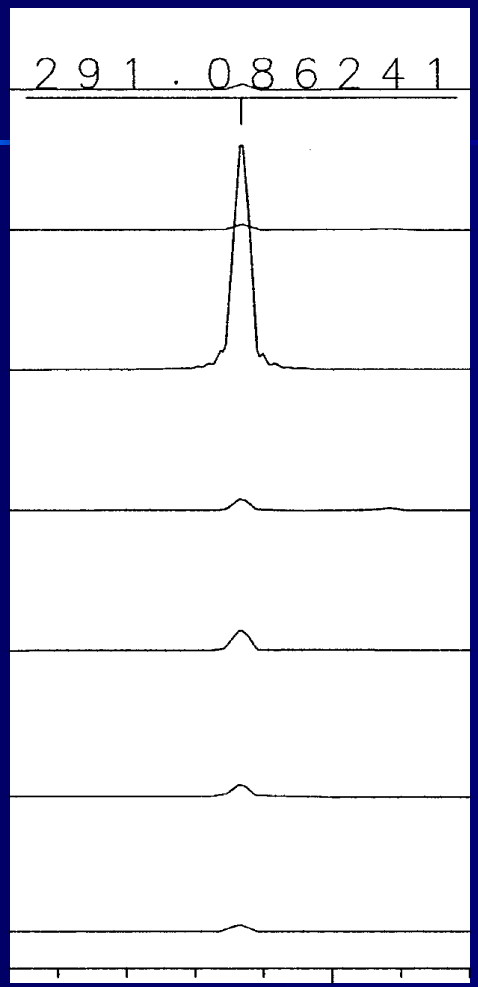
| Metabolite | CG | EF | NM | IM | EX | R/G | T/G | W/G |
|---|---|---|---|---|---|---|---|---|
| **Group 1: Early expression** | | **Profile A** | | | | | | |
| Ellagic acid* | P. Acid | $C_{14}H_6O_8$ | 302.0063 | AP- | AN | 0.03 | 0.03 | 0.03 |
| Hydroxybenzoic acid* | P. Acid | $C_7H_6O_3$ | 138.0317 | AP- | AN | 0.09 | 0.08 | 0.09 |
| Arginine | A. acid | $C_6H_{14}N_4O_2$ | 174.1117 | ES+ | 50 | 0.06 | 0.06 | 0.10 |
| Vanillic acid* | P. Acid | $C_8H_8O_4$ | 168.0423 | AP- | AN | 0.08 | 0.05 | 0.06 |
| Malic acid | O. acid | $C_4H_6O_5$ | 134.0215 | ES- | AN | 0.04 | 0.04 | 0.04 |
| Catechin (epi)/leucopelargonidin* | Phenolic | $C_{15}H_{14}O_6$ | 290.0790 | ES- | 50 | 0.04 | 0.07 | 0.12 |
| Caffeate* | P. Acid | $C_9H_8O_4$ | 180.0421 | AP- | 50 | 0.16 | 0.14 | 0.11 |
| Ethyl cinnamate* | Ester | $C_{11}H_{12}O_2$ | 176.0837 | AP- | AN | 0.13 | 0.13 | 0.12 |
| Quercitin glucoside* | Flavonoid | $C_{21}H_{20}O_{12}$ | 464.0955 | ES- | 50 | 0.13 | 0.13 | 0.13 |
| Methyl cinnamate* | Ester | $C_{10}H_{10}O_2$ | 162.0681 | AP- | AN | 0.19 | 0.1 | 0.14 |
| Dihydrokaempferol* | Flavonoid | $C_{15}H_{12}O_6$ | 288.0634 | AP+ | 50 | 0.11 | 0.15 | 0.28 |
| Leucocyanidin* | Flavonoid | $C_{15}H_{14}O_7$ | 306.0737 | AP- | AN | 0.37 | 0.37 | 0.37 |
| *N*-Alanylcysteine | A. Acid d. | $C_6H_{12}N_2O_3S$ | 192.0567 | ES+ | AN | 0.22 | 0.22 | 0.22 |
| Gentisic/Protocatechuic acid* | P. Acid | $C_7H_6O_4$ | 154.0266 | AP+ | 50 | 0.31 | 0.26 | 0.25 |
| Malusfuran | Furan | $C_{12}H_8O_5$ | 232.0370 | AP- | 50 | 0.25 | 0.25 | 0.25 |
| (E)2-hexenol / hexanal | Alcohol | $C_6H_{12}O$ | 100.0888 | AP+ | AN | 0.25 | 0.25 | 0.25 |
| Glucogallin* | Phenolic | $C_{13}H_{16}O_{10}$ | 332.0744 | AP- | 50 | 0.23 | 0.23 | 0.23 |
| Pantothenic acid | Vitamin | $C_9H_{17}NO_5$ | 219.1107 | ES+ | AN | 0.27 | 0.27 | 0.27 |
| Dihydroxybenzoquinone | Phenolic | $C_6H_4O_4$ | 140.0109 | ES+ | AN | 0.49 | 0.48 | 0.46 |
| Dihydroquercitin* | Flavonoid | $C_{15}H_{12}O_7$ | 304.0581 | AP+ | 50 | 0.42 | 0.42 | 0.42 |

# Metabolic profiling of strawberry fruit development

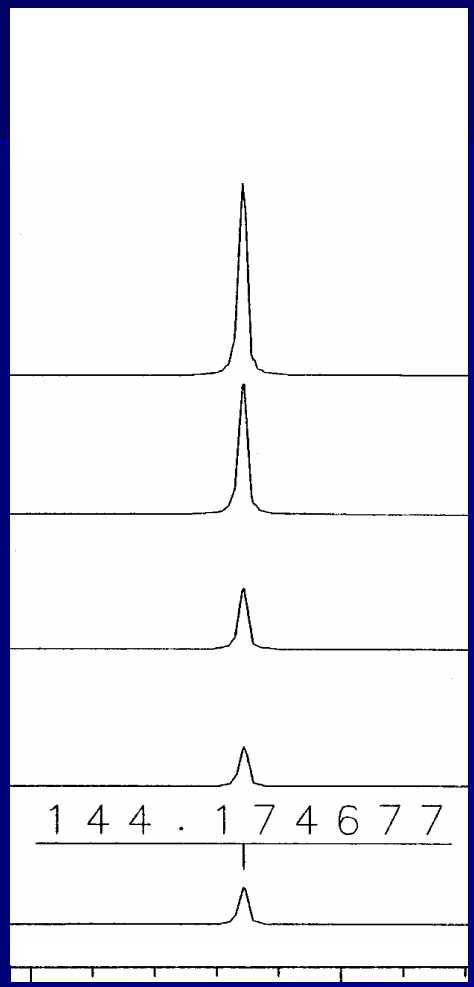
| Metabolite | CG | EF | NM | IM | EX | R/G | T/G | W/G |
|---|---|---|---|---|---|---|---|---|
| | | | **Profile I** | | | | | |
| Linoleic acid | F. Acid | $C_{18}H_{32}O_2$ | 280.2402 | AP+ | AN | **20.80** | 1.93 | 0.97 |
| Palmitoleic acid | F. Acid | $C_{16}H_{30}O_2$ | 254.2246 | AP+ | AN | **24.30** | 1.34 | 0.85 |
| Oleic acid | F. Acid | $C_{18}H_{34}O_2$ | 282.2559 | AP+ | AN | **32.56** | 1.50 | 0.99 |
| Pentadecanoic acid | F. Acid | $C_{15}H_{30}O_2$ | 242.2246 | AP+ | AN | **28.80** | 0.91 | 0.62 |
| Palmitic acid | F. Acid | $C_{16}H_{32}O_2$ | 256.2402 | AP+ | AN | **40.20** | 1.67 | 0.80 |
| 3-methylbutyl nonanoate | Ester | $C_{14}H_{28}O_2$ | 228.2089 | AP+ | AN | **29.80** | 1.38 | 1.00 |
| Stearic acid | F. acid | $C_{18}H_{36}O_2$ | 284.2715 | AP+ | AN | **18.03** | 1.87 | 1.24 |
| Furaneol (R=D-glucopyranose) | Furan | $C_{12}H_{18}O_8$ | 290.1002 | ES+ | AN | **10.04** | 1.00 | 1.00 |
| Furaneol (R=D-glucopyranose, malonyl) | Furan | $C_{15}H_{20}O_{11}$ | 376.1006 | ES+ | 50 | **11.00** | 1.00 | 1.00 |
| Nonanoic acid | F. Acid | $C_9H_{18}O_2$ | 158.1307 | AP+ | AN | **5.20** | 1.00 | 1.00 |
| Capric acid | F. Acid | $C_{10}H_{20}O_2$ | 172.1463 | AP+ | AN | **5.49** | 1.00 | 1.00 |
| Eicosanoic acid | F. Acid | $C_{20}H_{40}O_2$ | 312.3028 | AP+ | AN | **7.85** | 1.00 | 1.00 |
| Linolenic acid | F. Acid | $C_{18}H_{30}O_2$ | 278.2246 | AP+ | AN | **8.52** | 1.97 | 1.10 |
| Cinnamate* | P. Acid | $C_9H_8O_2$ | 148.0524 | AP+ | 50 | **5.86** | 1.17 | 0.87 |
| 1-octyl butanoate | Ester | $C_{12}H_{24}O_2$ | 200.1776 | AP+ | AN | **5.69** | 1.00 | 1.00 |
| 1-methyhexyl hexanoate | Ester | $C_{13}H_{26}O_2$ | 214.1933 | AP+ | AN | **6.67** | 1.00 | 1.00 |
| Cis-hex-3-enyl octanoate | Ester | $C_{14}H_{26}O_2$ | 226.1933 | AP+ | AN | **6.93** | 0.91 | 0.90 |
| Methylstearate | Ester | $C_{19}H_{38}O_2$ | 298.2872 | AP+ | AN | **7.91** | 0.76 | 0.68 |
| Sinapyl alcohol* | Phenolic | $C_{11}H_{14}O_4$ | 210.0891 | AP- | AN | **5.8** | 1.00 | 1.00 |
| Scopoletin* | Benzopyranoid | $C_{10}H_8O_4$ | 192.0421 | AP+ | 50 | **5.31** | 1.94 | 0.99 |
| 2-Isopropyl-3-methoxypyrazine | Alkaloid | $C_8H_{12}N_2O$ | 152.0949 | AP+ | AN | **6.79** | 1.00 | 1.00 |
| Farnesyl acetone | Terpene | $C_{18}H_{30}O$ | 262.2295 | AP+ | AN | **6.65** | 1.57 | 0.59 |
| Hydroxycoumarine* | Benzopyranoid | $C_9H_6O_3$ | 162.0316 | AP+ | AN | **5.44** | 0.73 | 0.66 |
| Coumarine* | Benzopyranoid | $C_9H_6O_2$ | 146.0368 | AP+ | AN | **5.47** | 0.96 | 1.57 |
| Pelargonidin* | Flavonoid | $C_{15}H_{10}O_5$ | 270.0527 | ES- | 50 | **9.46** | 1.00 | 1.00 |
| Smipine | Alkaloid | $C_{10}H_{16}N_2O$ | 180.1261 | AP+ | AN | **5.33** | 1.00 | 1.00 |

# Group 1: Early expression

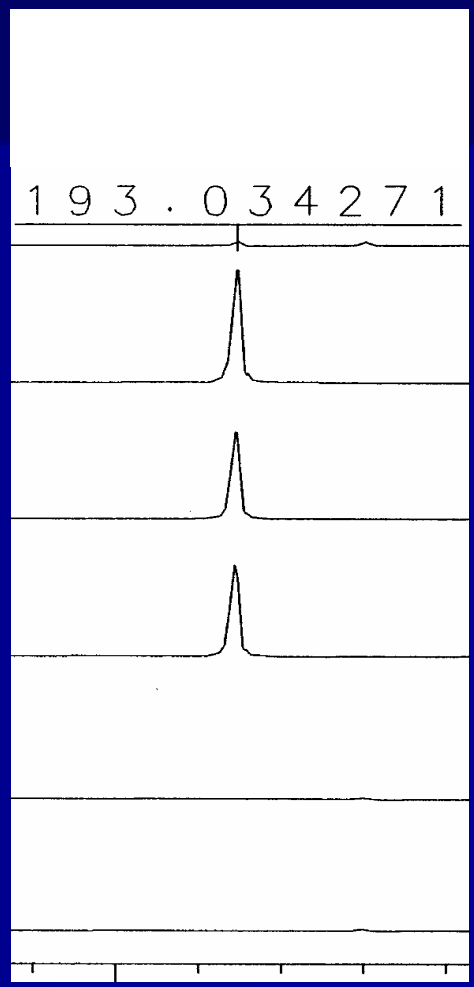





Catechin
(APCI +)

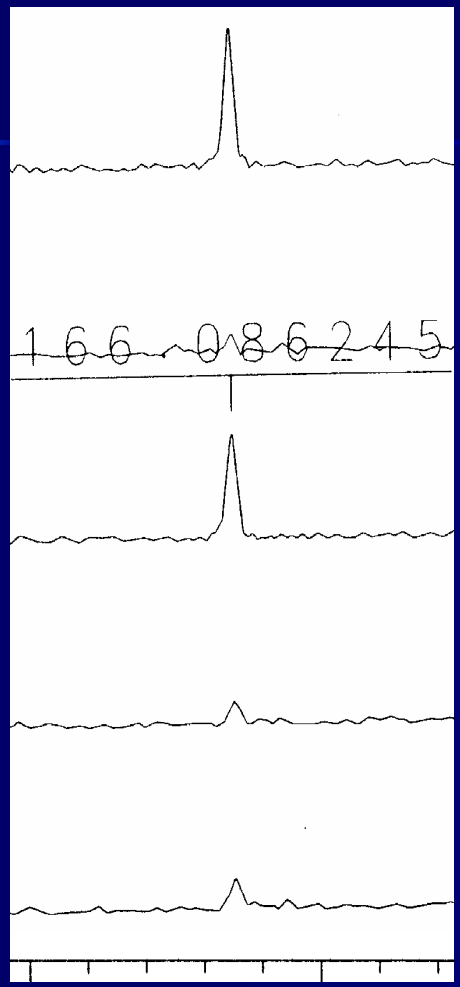



Heptylamine
(ESI+)

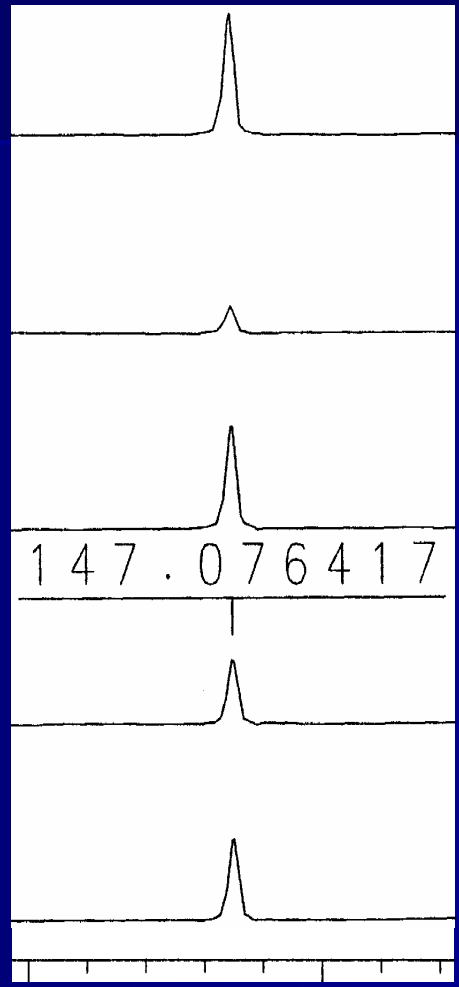



Citrate/Isocitrate
(APCI+)

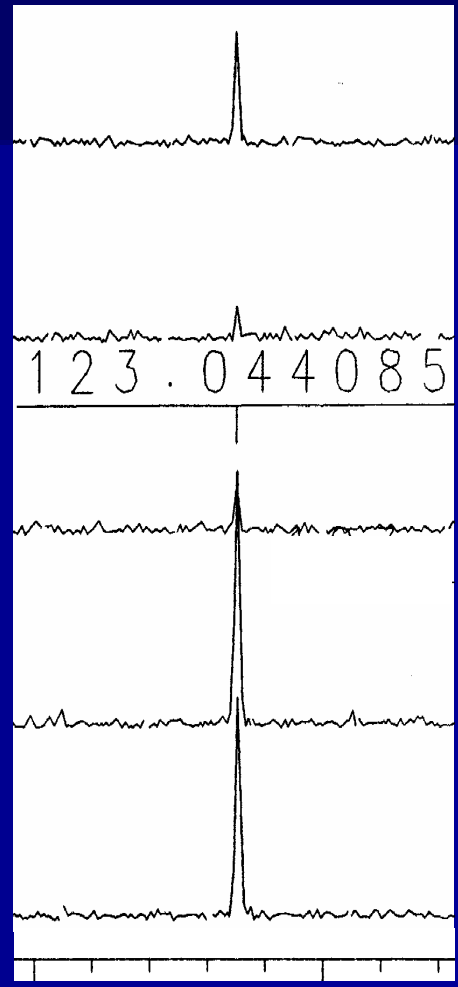
# Group 2: Intermediate expression




Phenylalanine
(ESI+)

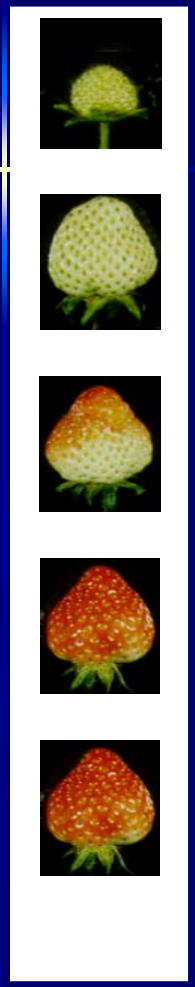


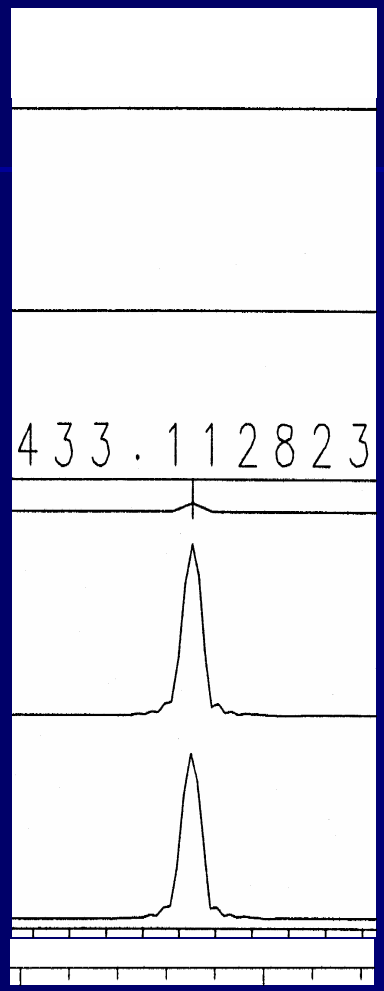

Glutamine
(ESI+)



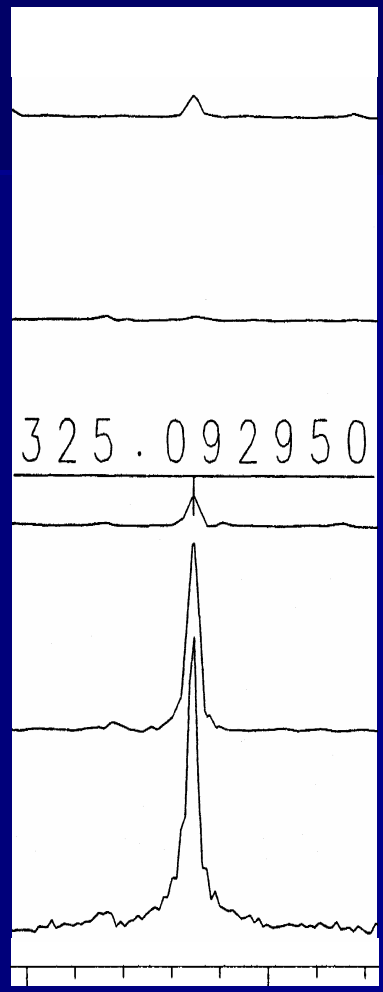

Benzoate
(APCI+)

# Group 3: Late expression

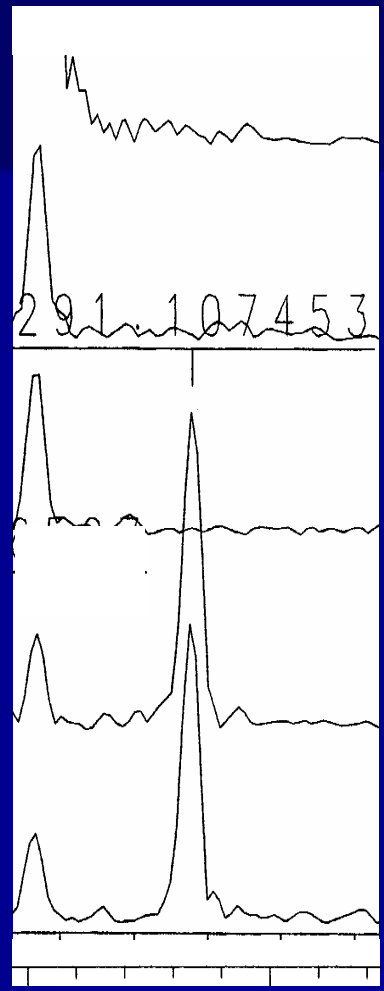




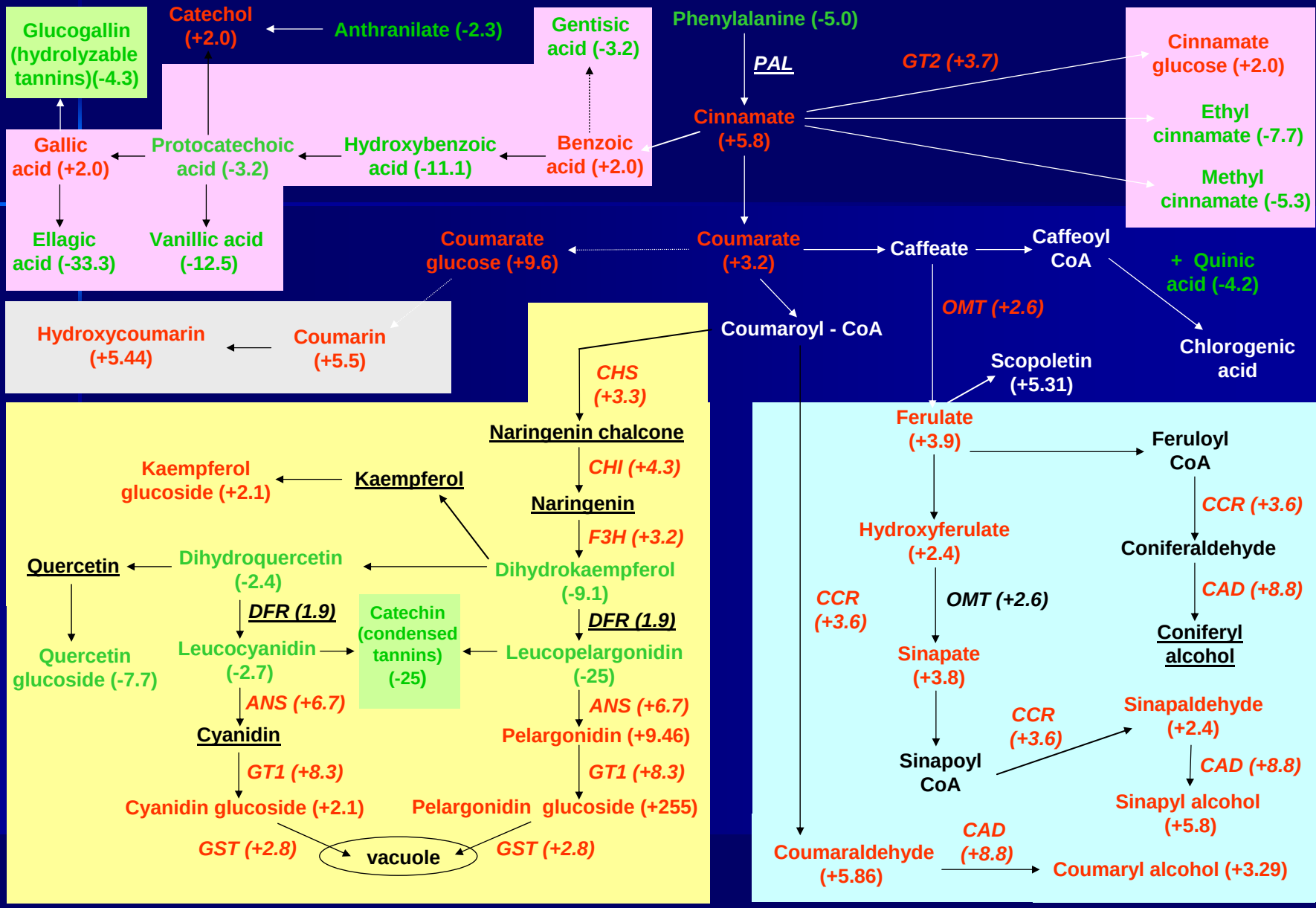

Pelargonidin glucoside (ESI+)



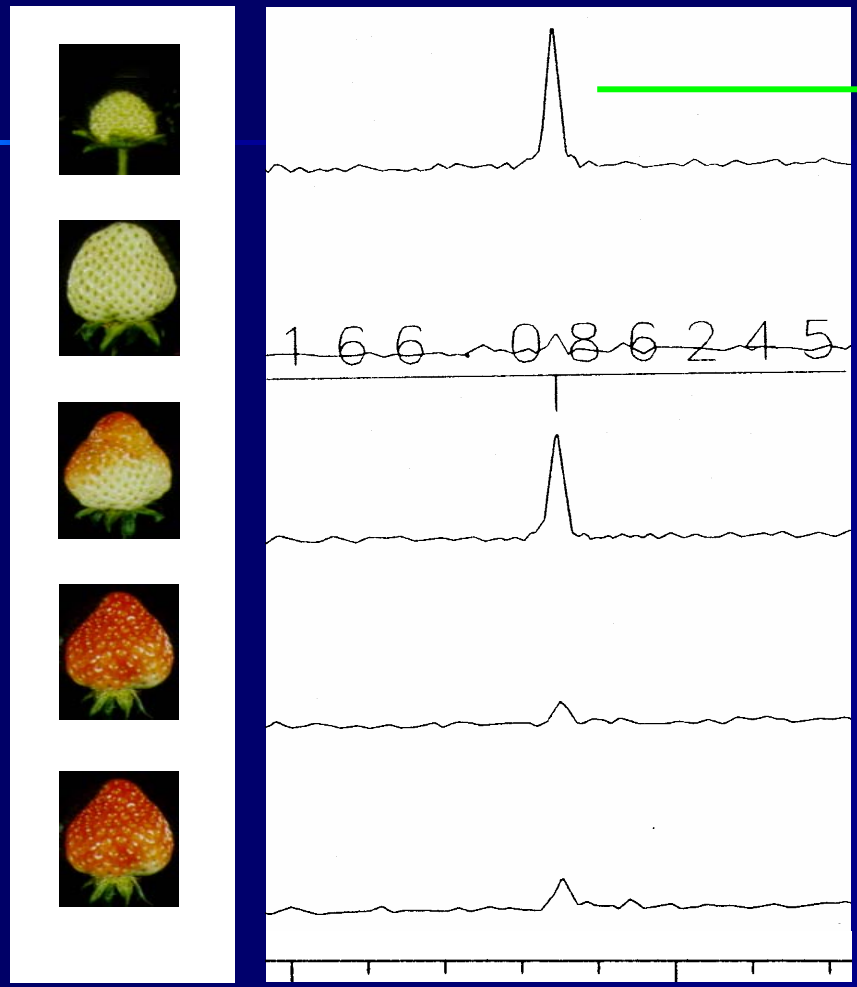

Coumaroyl glucoside (ESI-)

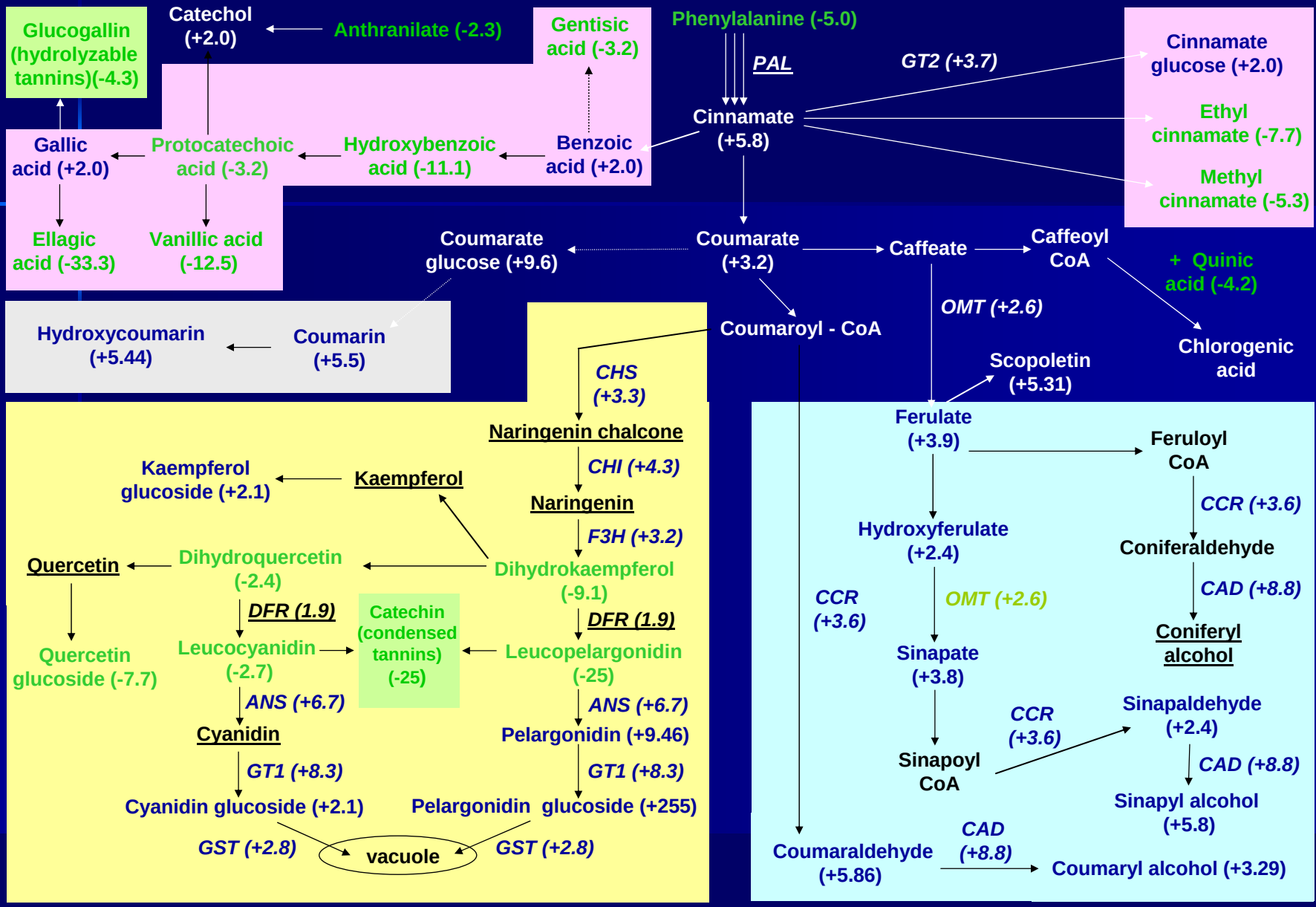



Furaneol glucoside (ESI+)

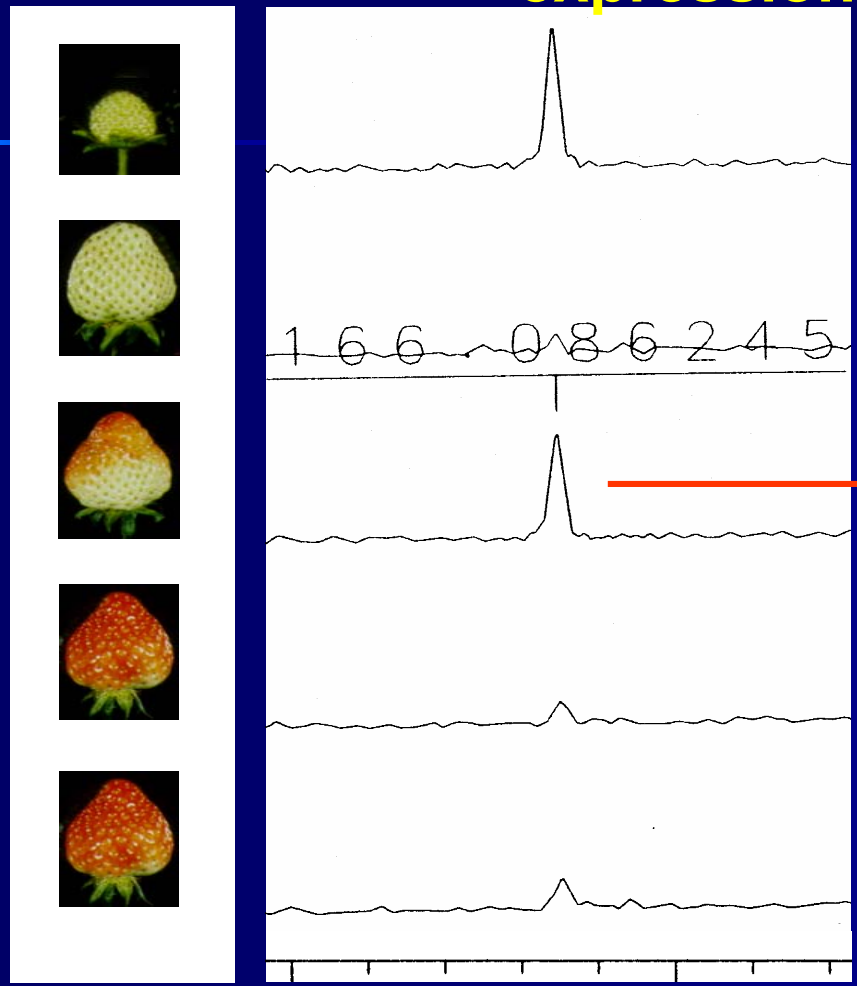

Glucogallin (hydrolyzable tannins)(-4.3)
Catechol (+2.0)
Anthranilate (-2.3)
Gentisic acid (-3.2)
Phenylalanine (-5.0)
PAL
GT2 (+3.7)
Cinnamate glucose (+2.0)
Ethyl cinnamate (-7.7)
Methyl cinnamate (-5.3)
Cinnamate (+5.8)
Gallic acid (+2.0)
Protocatechoic acid (-3.2)
Hydroxybenzoic acid (-11.1)
Benzoic acid (+2.0)
Ellagic acid (-33.3)
Vanillic acid (-12.5)
Coumarate glucose (+9.6)
Coumarate (+3.2)
Caffeate
Caffeoyl CoA
+ Quinic acid (-4.2)
Chlorogenic acid
OMT (+2.6)
Coumaroyl - CoA
Hydroxycoumarin (+5.44)
Coumarin (+5.5)
CHS (+3.3)
Scopoletin (+5.31)
Naringenin chalcone
CHI (+4.3)
Kaempferol glucoside (+2.1)
Kaempferol
Naringenin
F3H (+3.2)
Quercetin
Dihydroquercetin (-2.4)
Dihydrokaempferol (-9.1)
DFR (1.9)
DFR (1.9)
Quercetin glucoside (-7.7)
Leucocyanidin (-2.7)
Catechin (condensed tannins) (-25)
Leucopelargonidin (-25)
ANS (+6.7)
ANS (+6.7)
Cyanidin
Pelargonidin (+9.46)
GT1 (+8.3)
GT1 (+8.3)
Cyanidin glucoside (+2.1)
Pelargonidin glucoside (+255)
GST (+2.8)
vacuole
GST (+2.8)
Ferulate (+3.9)
Feruloyl CoA
CCR (+3.6)
Hydroxyferulate (+2.4)
Coniferaldehyde
CAD (+8.8)
CCR (+3.6)
OMT (+2.6)
Coniferyl alcohol
Sinapate (+3.8)
CCR (+3.6)
Sinapaldehyde (+2.4)
Sinapoyl CoA
CAD (+8.8)
Sinapyl alcohol (+5.8)
CAD (+8.8)
Coumaraldehyde (+5.86)
Coumaryl alcohol (+3.29)

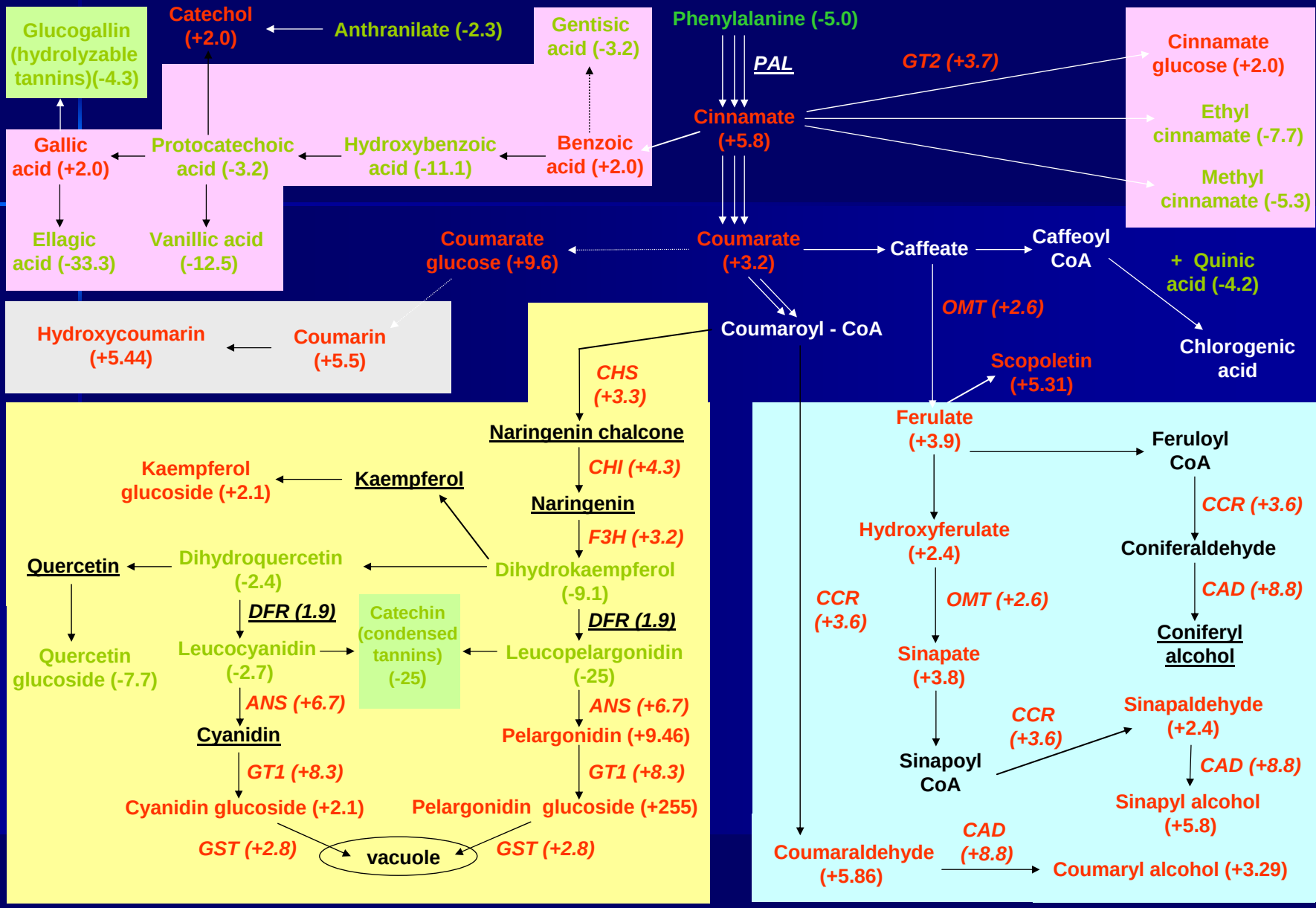
# Phenylalanine and changes in gene and metabolite expression



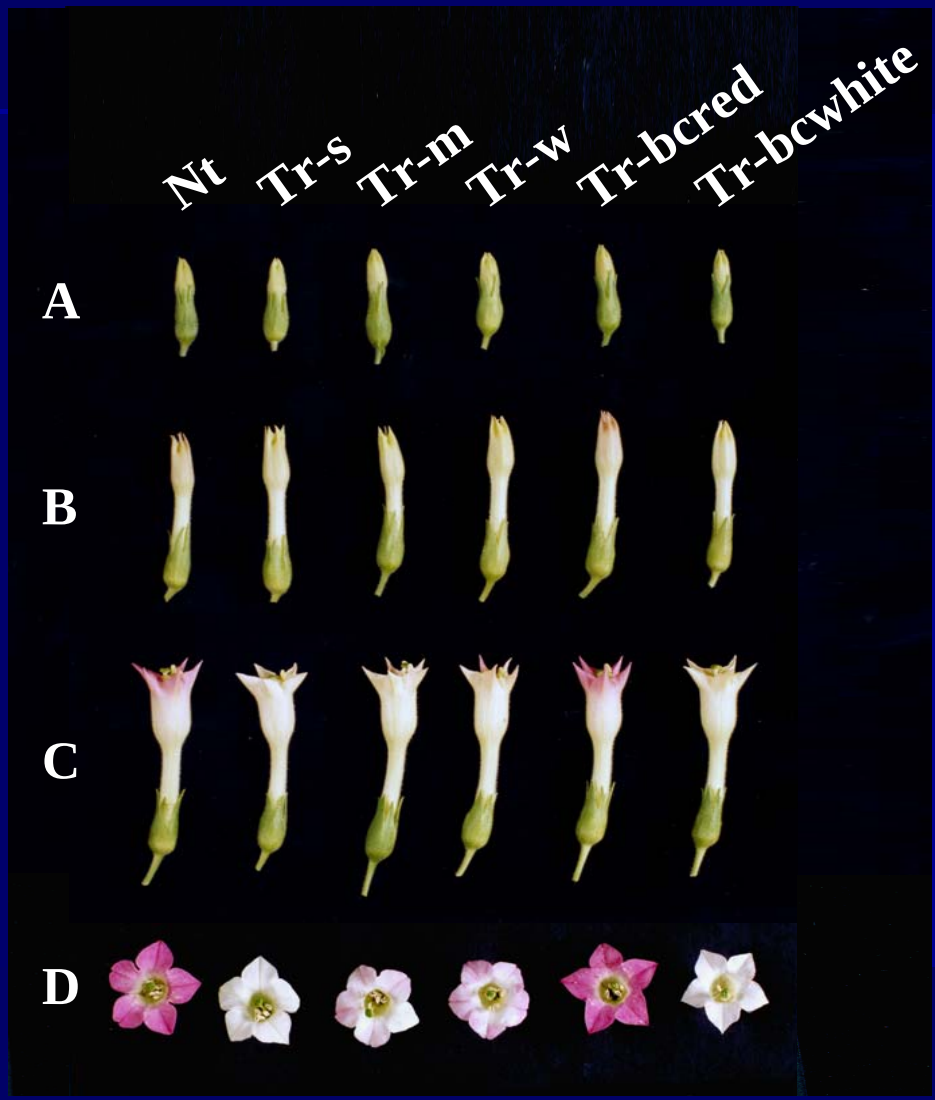

Glucogallin (hydrolyzable tannins)(-4.3)
Catechol (+2.0)
Anthranilate (-2.3)
Gentisic acid (-3.2)
Phenylalanine (-5.0)
PAL
GT2 (+3.7)
Cinnamate glucose (+2.0)
Ethyl cinnamate (-7.7)
Methyl cinnamate (-5.3)
Cinnamate (+5.8)
Gallic acid (+2.0)
Protocatechoic acid (-3.2)
Hydroxybenzoic acid (-11.1)
Benzoic acid (+2.0)
Ellagic acid (-33.3)
Vanillic acid (-12.5)
Coumarate glucose (+9.6)
Coumarate (+3.2)
Caffeate
Caffeoyl CoA
+ Quinic acid (-4.2)
Chlorogenic acid
OMT (+2.6)
Coumaroyl - CoA
Hydroxycoumarin (+5.44)
Coumarin (+5.5)
CHS (+3.3)
Scopoletin (+5.31)
Naringenin chalcone
CHI (+4.3)
Kaempferol glucoside (+2.1)
Kaempferol
Naringenin
F3H (+3.2)
Quercetin
Dihydroquercetin (-2.4)
Dihydrokaempferol (-9.1)
DFR (1.9)
DFR (1.9)
Quercetin glucoside (-7.7)
Leucocyanidin (-2.7)
Catechin (condensed tannins) (-25)
Leucopelargonidin (-25)
ANS (+6.7)
ANS (+6.7)
Cyanidin
Pelargonidin (+9.46)
GT1 (+8.3)
GT1 (+8.3)
Cyanidin glucoside (+2.1)
Pelargonidin glucoside (+255)
GST (+2.8)
vacuole
GST (+2.8)
Ferulate (+3.9)
Feruloyl CoA
CCR (+3.6)
Hydroxyferulate (+2.4)
Coniferaldehyde
CAD (+8.8)
CCR (+3.6)
OMT (+2.6)
Coniferyl alcohol
Sinapate (+3.8)
CCR (+3.6)
Sinapaldehyde (+2.4)
Sinapoyl CoA
CAD (+8.8)
Sinapyl alcohol (+5.8)
CAD (+8.8)
Coumaraldehyde (+5.86)
Coumaryl alcohol (+3.29)

# Phenylalanine and changes in gene and metabolite expression

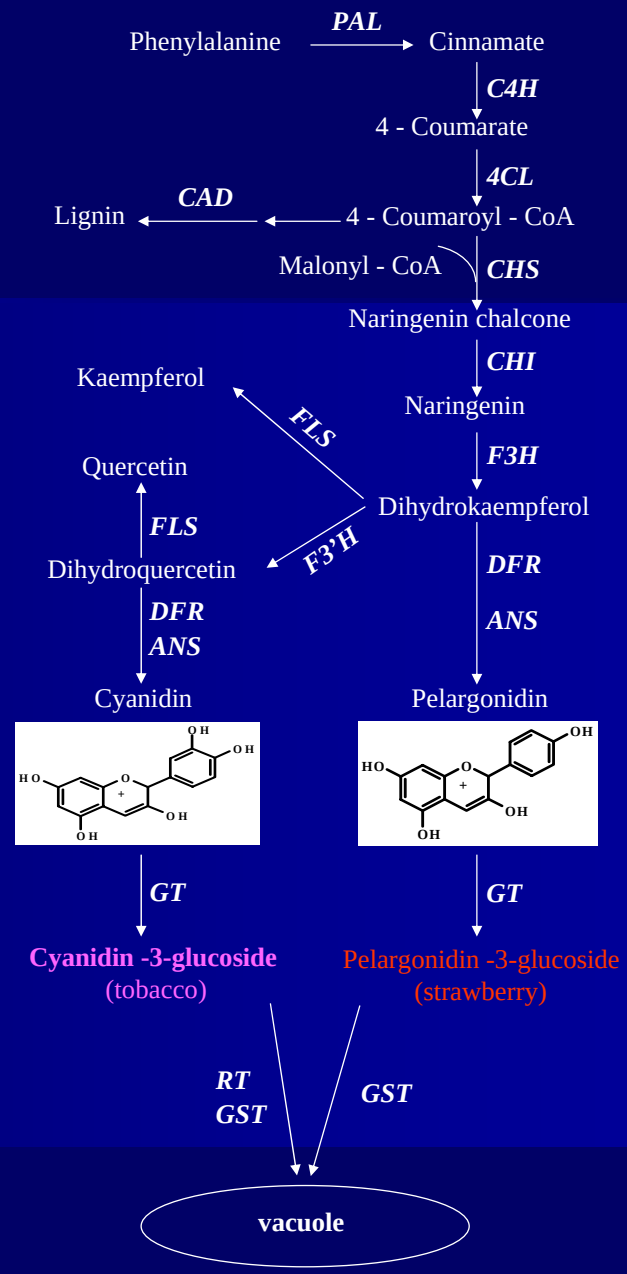



Glucogallin (hydrolyzable tannins)(-4.3)
Catechol (+2.0)
Anthranilate (-2.3)
Gentisic acid (-3.2)
Phenylalanine (-5.0)
PAL
GT2 (+3.7)
Cinnamate glucose (+2.0)
Ethyl cinnamate (-7.7)
Methyl cinnamate (-5.3)
Cinnamate (+5.8)
Gallic acid (+2.0)
Protocatechoic acid (-3.2)
Hydroxybenzoic acid (-11.1)
Benzoic acid (+2.0)
Ellagic acid (-33.3)
Vanillic acid (-12.5)
Coumarate glucose (+9.6)
Coumarate (+3.2)
Caffeate
Caffeoyl CoA
+ Quinic acid (-4.2)
Chlorogenic acid
OMT (+2.6)
Coumaroyl - CoA
Hydroxycoumarin (+5.44)
Coumarin (+5.5)
CHS (+3.3)
Scopoletin (+5.31)
Naringenin chalcone
CHI (+4.3)
Kaempferol glucoside (+2.1)
Kaempferol
Naringenin
F3H (+3.2)
Quercetin
Dihydroquercetin (-2.4)
Dihydrokaempferol (-9.1)
DFR (1.9)
DFR (1.9)
Quercetin glucoside (-7.7)
Leucocyanidin (-2.7)
Catechin (condensed tannins) (-25)
Leucopelargonidin (-25)
ANS (+6.7)
ANS (+6.7)
Cyanidin
Pelargonidin (+9.46)
GT1 (+8.3)
GT1 (+8.3)
Cyanidin glucoside (+2.1)
Pelargonidin glucoside (+255)
GST (+2.8)
vacuole
GST (+2.8)
Ferulate (+3.9)
Feruloyl CoA
CCR (+3.6)
Hydroxyferulate (+2.4)
Coniferaldehyde
CAD (+8.8)
CCR (+3.6)
OMT (+2.6)
Coniferyl alcohol
Sinapate (+3.8)
CCR (+3.6)
Sinapaldehyde (+2.4)
Sinapoyl CoA
CAD (+8.8)
Sinapyl alcohol (+5.8)
CAD (+8.8)
Coumaraldehyde (+5.86)
Coumaryl alcohol (+3.29)

# *Over-expression of FaMYB1 in Tobacco*



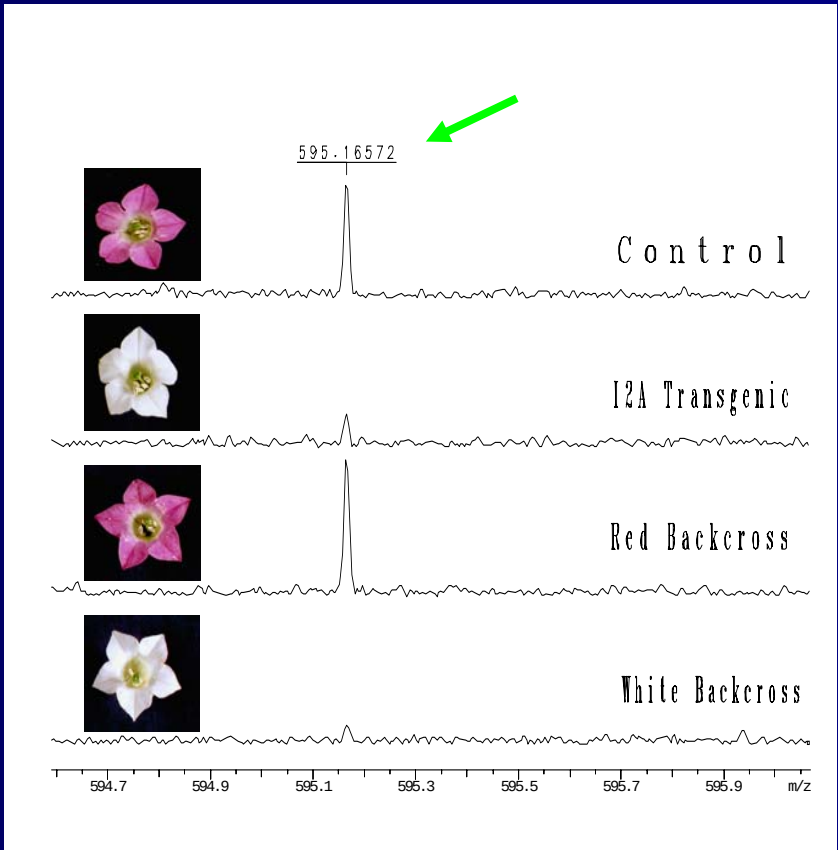
# The phenylpropanoid pathway in strawberry & tobacco



# Differentially Expressed Masses Between Wild-Type and Transgenic Flowers

| No. | IM | OM | T1-white | Bc-red | Bc-white |
|---|---|---|---|---|---|
| 1 | APCI- | 164.0717 | ↑[a] | = | ↑ |
| 2 | APCI- | 273.0768 | ↓ | = | ↓ |
| 3 | APCI- | 289.0717 | ↓ | = | ↓ |
| 4 | APCI- | 363.1658 | ↑ | = | ↑ |
| 5 | APCI- | 435.4205 | ↑ | = | ↑ |
| 6 | APCI- | 463.4522 | ↑ | = | ↑ |
| 7 | APCI+ | 486.2601 | ↑ | = | ↑ |
| 8 | ESI- | 257.0954 | ↓ | = | ↓ |
| 9 | ESI+ | 595.1657 | ↓ | = | ↓ |

# Wild type vs. White Transgenic Tobacco (mass no. 9)




**Observed mass of unknown:**
**595.16572**

**Empirical formula search result (1 possibility only):**
**$C_{27}H_{30}O_{15}$ [+H]+**

**Mass:**
**595.16575**

**Mass error:**
**0.04 ppm**

DICTIONARY OF NATURAL PRODUCTS

Chemical Databases Online

# 18 Possibilities in Search of the Natural Product Database for C27H30O15:

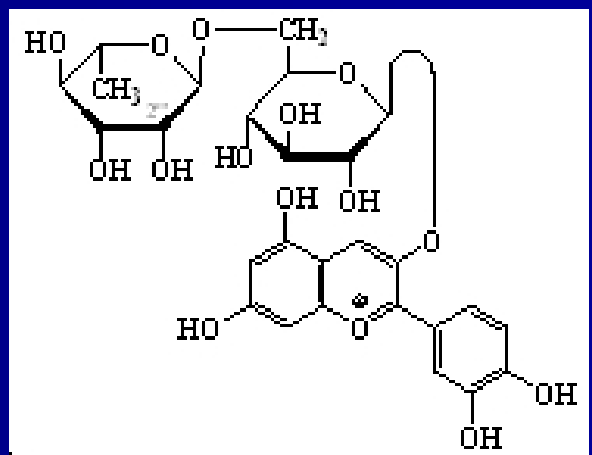
| Name | CAS Registry Number | Molecular Formula |
|---|---|---|
| **Keracyanin, CI, INN8** | 18719-76-1 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,3',4',5,7-Pentahydroxyflavylium(1+); 3-glucopyranoside-D-β-*O*-5Rhamnoside, -L-α-*O* | 53859-12-4 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,3',4',5,7-Pentahydroxyflavylium(1+); 3-]galactopyranoside-D-(4→1)-Rhamnopyranosyl-L-α]-*O* | 54542-59-5 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,3',4',5,7-Pentahydroxyflavylium(1+); 3-]galactopyranoside-D-β-(6→1)-Rhamnopyranosyl-L-α]-*O* | 50816-67-6 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,3',4',5,7-Pentahydroxyflavylium(1+); 3-]glucopyranoside-D-β-(2→1)-Rhamnopyranosyl-L-α]-*O* | 54947-80-7 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,4',5,6,7-Pentahydroxyflavylium(1+); 3-]glucopyranoside-D-β-(6→1)-Rhamnopyranosyl-L-α]-*O* | | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,4',5,7-Tetrahydroxyflavylium(1+), glucopyranoside-D-β-*O*-Di-3,5 ;CI8 | 17334-58-6 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,4',5,7-Tetrahydroxyflavylium(1+), CI8, 3-]glucopyranoside-D-β-(2→1)-Glucopyranosyl-D-β]-*O* | 54542-60-8 | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3,4',5,7-Tetrahydroxyflavylium(1+), CI8, 3-]glucopyranoside-D-β-(6→1)-Glucopyranosyl-D-β]-*O* | | $C_{27}H_{31}O_{15}^{(+)}$ |
| 3',4',5,7-Tetrahydroxyflavylium(1+); 5,7-Di-glucopyranoside-D-β-*O* | 53947-98-1 | $C_{27}H_{31}O_{15}^{(+)}$ |

DICTIONARY OF NATURAL PRODUCTS

Chemical Databases Online

All 18 Possibilities for C27H30O15 are glycosides of either Cyandin, Pelargonidin, Peonidin or Luteolidin

One of the candidates, Keracyanin (cyanidn-3-rhamnoside) was reported in 1992 to accmulate in Tobbaco flowers.



**Entry Name:** Keracyanin, 8CI, INN **Synonym(s):** 3-*O*-Rhamnoglucosyloxycyanidin. Ceracyanin. Sambucin. Antirrhinin. Cyanidin 3-rutinoside. Cyaninoside. Meralop. Noctilux. Nyctalux. Prunicyanin. Oleocyanin

**Chapman & Hall Number:** BFH51-Q

**CAS Registry Number:** 18719-76-1

**Type of Compound Code(s):** VK0050

**Molecular Formula:** $C_{27}H_{31}O_{15}^{(+)}$

**Molecular Weight:** 595.533

**Accurate Mass:** 595.1663

**Percentage Composition:** C 54.45%; H 5.25%; O 40.30%

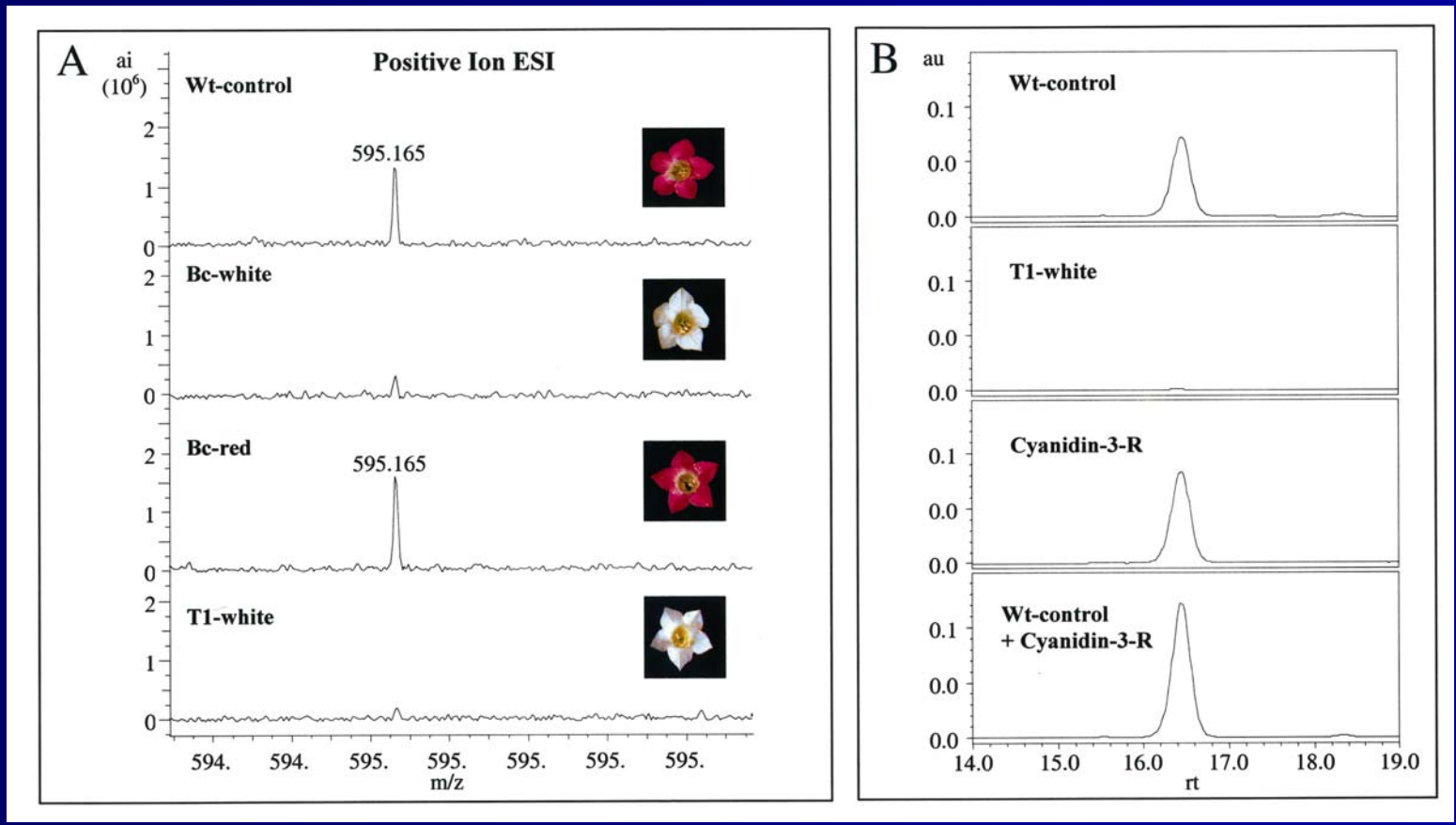
**General Statement:** Glycoside of 3,3',4',5,7-Pentahydroxyflavylium(1+) CMQ73-Z

**Biological Source:** Pigment present in fresh blossoms of *Canna generalis* and *Silene dioica*, as well as in *Prunus*, *Viola*, *Tulipa* and other plants

**Biological Use / Importance:** Improves vision in humans (for night blindness)

**Physical Description:** Fine red needles (dil. HCl) (as chloride) **Melting Point:** Mp 175° (chloride) **Other Data:** CAS no. refers to chloride

# Confirmation of Cyandin 3- Rhamnoglucoside in Mass no. 9)



# Enhancing the Data Obtained by High Resolution MS Using Isotope Abundune

**Metabolomic database annotations via query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm**

*BMC Bioinformatics* 2006, **7**:234 doi:10.1186/1471-2105-7-234

Tobias Kind (tkind@ucdavis.edu)
Oliver Fiehn (ofiehn@ucdavis.edu)

# Using Abundance of Isotopes for Metabolite Elucidation (in Spectra)

*Atomic Masses and Abundances for a Subset of Naturally Occurring Biologically Relevant Isotopes*

| Iso | A | % | Iso | A+1 | % | Iso | A+2 | % | Iso | A+3 | % | Iso | A+4 | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $^{12}C$ | 12 | 98.93(8) | $^{13}C$ | 13.0033548378 (10) | 1.07(8) | $^{14}C$ | 14.003241988(4) | - | - | - | - | - | - | - |
| $^{1}H$ | 1.0078250321(4) | 99.9885(70) | $^{2}H$ | 2.0141017780(4) | 0.0115 (70) | $^{3}H$ | 3.0160492675(11) | - | - | - | - | - | - | - |
| $^{14}N$ | 14.0030740052(9) | 99.632(7) | $^{15}N$ | 15.0001088984(9) | 0.368(7) | . | - | - | - | - | - | - | - | - |
| $^{16}O$ | 15.9949146221(15) | 99.757(16) | $^{17}O$ | 16.99913150(22) | 0.038(1) | $^{18}O$ | 17.9991604(9) | 0.205(14) | - | - | - | - | - | - |
| $^{32}S$ | 31.97207069(12) | 94.93(31) | $^{33}S$ | 32.97145850(12) | 0.76(2) | $^{34}S$ | 33.96786683(11) | 4.29(28) | - | - | - | $^{36}S$ | 35.96708088(25) | 0.02(1) |
| | | | | | | | | | | | | | | |
| $^{19}F$ | 18.99840320(7) | 100 | - | - | - | - | - | - | - | - | - | - | - | - |
| $^{23}Na$ | 22.98976967(23) | 100 | - | - | - | - | - | - | - | - | - | - | - | - |
| $^{39}K$ | 38.9637069(3) | 93.2581(44) | $^{40}K$ | 39.96399867(29) | 0.0117(1) | $^{41}K$ | 40.96182597(28) | 6.7302(44) | - | - | - | - | - | - |
| $^{31}P$ | 30.97376151(20) | 100 | - | - | - | - | - | - | - | - | - | - | - | - |
| $^{35}Cl$ | 34.96885271(4) | 75.781(4) | - | - | - | $^{37}Cl$ | 36.96590260(5) | 24.22(4) | - | - | - | - | - | - |
| $^{55}Mn$ | 54.9380496(14) | 100 | - | - | - | - | - | - | - | - | - | - | - | - |
| $^{54}Fe$ | 53.9396148(14) | 5.845(35) | - | - | - | $^{56}Fe$ | 55.9349421(15) | 91.754(36) | $^{57}Fe$ | 56.9353987(15) | 2.119 (10) | $^{58}Fe$ | 57.9332805(15) | 0.282(4) |
| $^{63}Cu$ | 62.9296011(15) | 69.17(3) | - | - | - | $^{65}Cu$ | 64.9277937(19) | 30.83(3) | - | - | - | - | - | - |
| $^{79}Br$ | 78.9183376(20) | 50.69(7) | - | - | - | $^{81}Br$ | 80.916291(3) | 49.31(7) | - | - | - | - | - | - |
| $^{127}I$ | 126.904468(4) | 100 | - | - | - | - | - | - | - | - | - | - | - | - |

# Metabolite Annotation Scheme

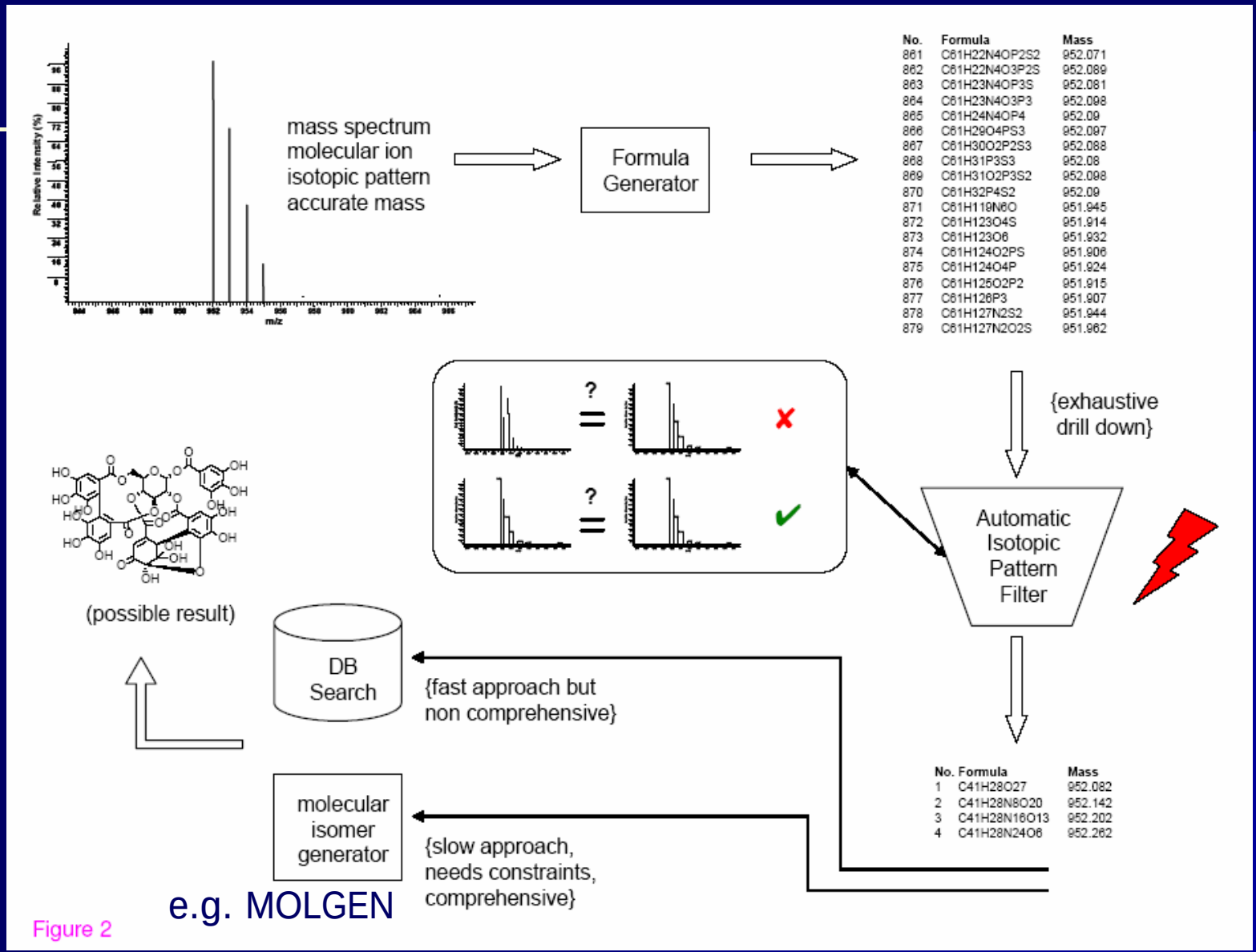



Figure 2

# Molecular Formula Search in Different Databases_

Table 1 - Example of a molecular formula search for $C_{15}H_{12}O_7$ in different chemical databases. Search date: July 2007.

| Database name | Compounds found | Total database entries |
|---|---|---|
| Chemical Abstracts (CAS) | 181 | 24,000,000 |
| Beilstein Database (MDL) | 166 | 8,000,000 |
| Dictionary of Natural Products (DNP) | 129 | 170,000 |
| PubChem (NIH) | 19 | 800,000 |
| Available Chemicals Directory (MDL) | 6 | 400,000 |
| ChemIDplus (NIH) | 6 | 370,000 |
| KEGG (Kyoto University) | 3 | 13,000 |
| NIST05 (NIST mass spectral database) | 2 | 163,000 |
| MOLGEN molecular isomer generator (allowing 2 benzene groups; 1 ether group, 1 keto group; 5 hydroxy groups) | 788,000 | - |

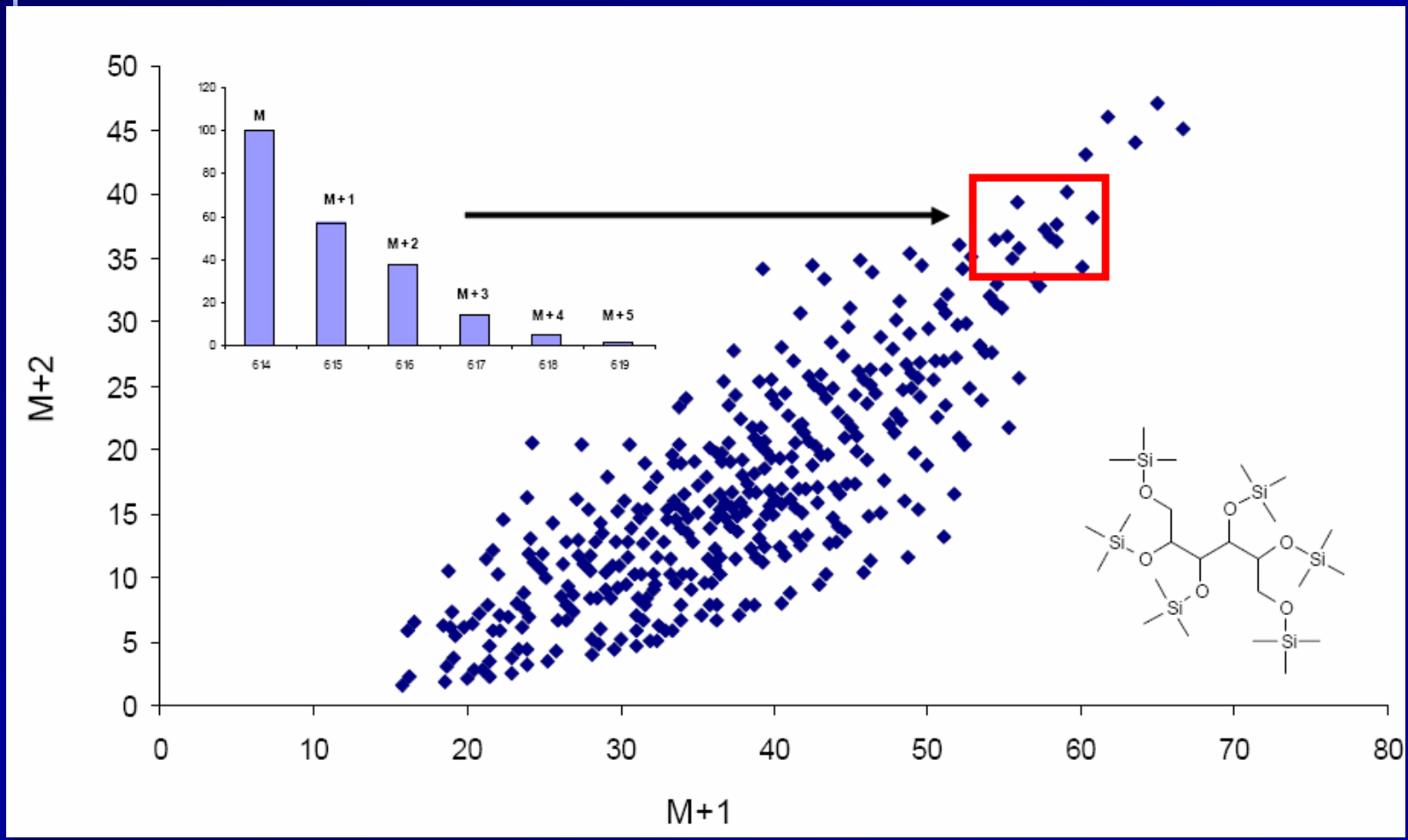
# Lower Mass Accuracy with Good Isotope Abundunce Analysis Compared to Higher Mass Accuracy and no Isotope Analysis

| | without isotope abundance information | | | | | 2% isotopic abundance accuracy | 5% isotopic abundance accuracy |
|---|---|---|---|---|---|---|---|
| molecular mass [Da] | 10 ppm | 5 ppm | 3 ppm | 1 ppm | 0.1 ppm | 3 ppm | 5 ppm |
| 150 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 200 | 3 | 2 | 2 | 1 | 1 | 1 | 1 |
| 300 | 24 | 11 | 7 | 2 | 1 | 1 | 6 |
| 400 | 78 | 37 | 23 | 7 | 1 | 2 | 13 |
| 500 | 266 | 115 | 64 | 21 | 2 | 3 | 33 |
| 600 | 505 | 257 | 155 | 50 | 5 | 4 | 36 |
| 700 | 1046 | 538 | 321 | 108 | 10 | 10 | 97 |
| 800 | 1964 | 973 | 599 | 200 | 20 | 13 | 111 |
| 900 | 3447 | 1712 | 1045 | 345 | 32 | 18 | 196 |

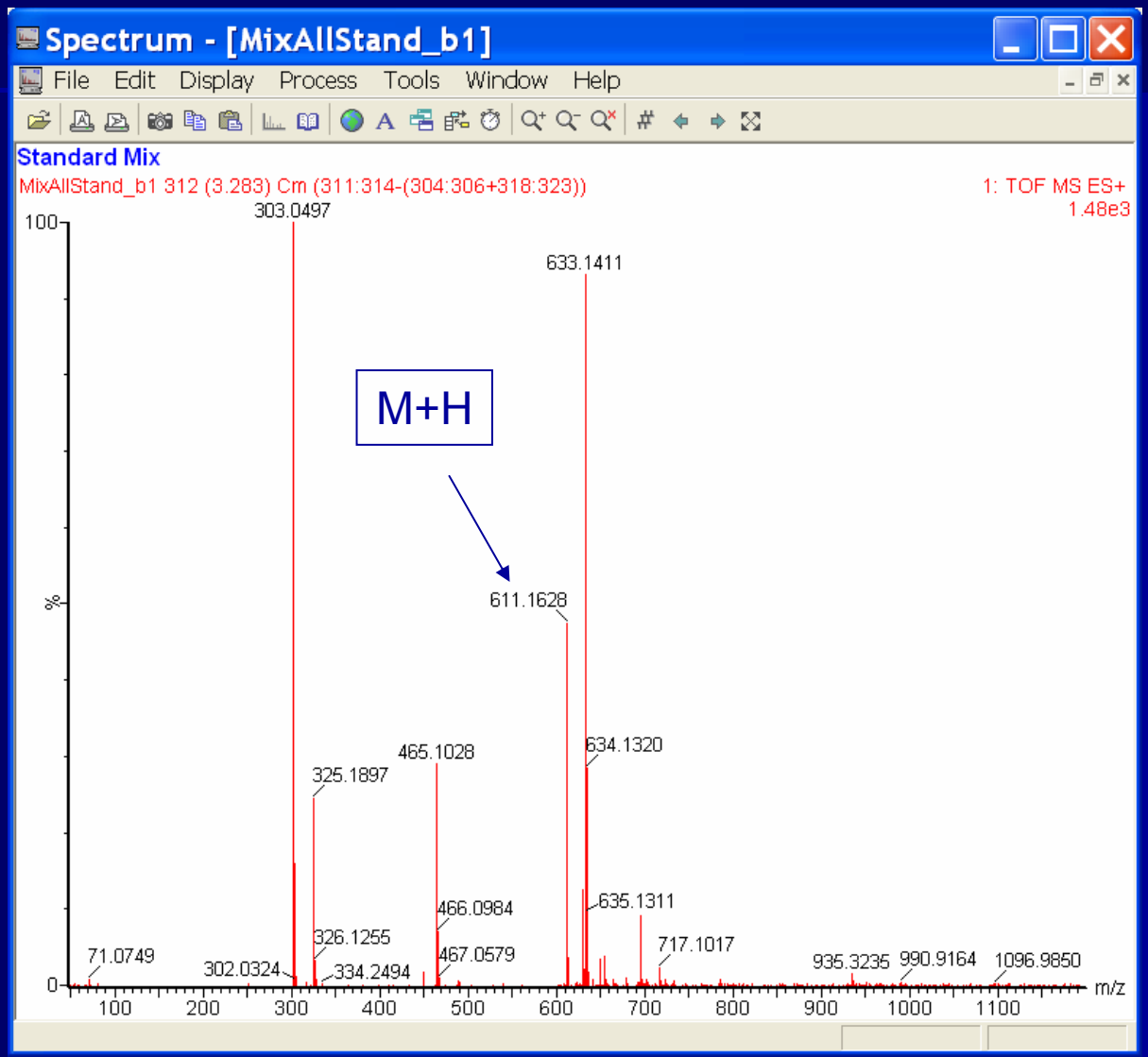
## The Conclusion from the Study

A Mass spectrometer capable of 3ppm mass accuracy and 2% error for isotopic abundunce patterns outperforms mass spectrometers with less than 1ppm mass accuaracy that does not include isotope information in the calculation of molecular formulae

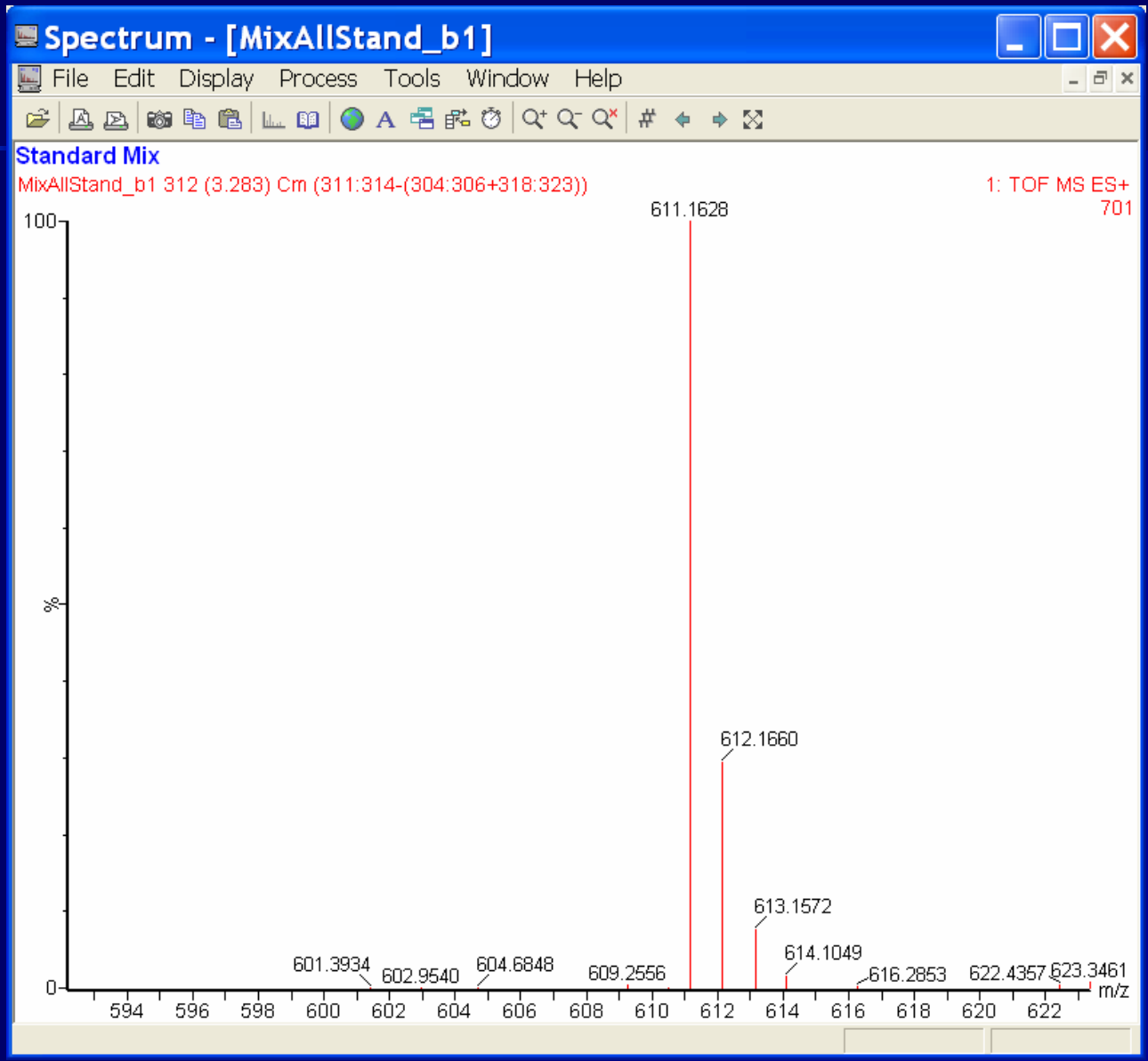
# Silylated Sorbitol (5% error on isotopic abundances)



# Full spectrum of Rutin standard



# Zoom on the Isotope pattern of 611.1628



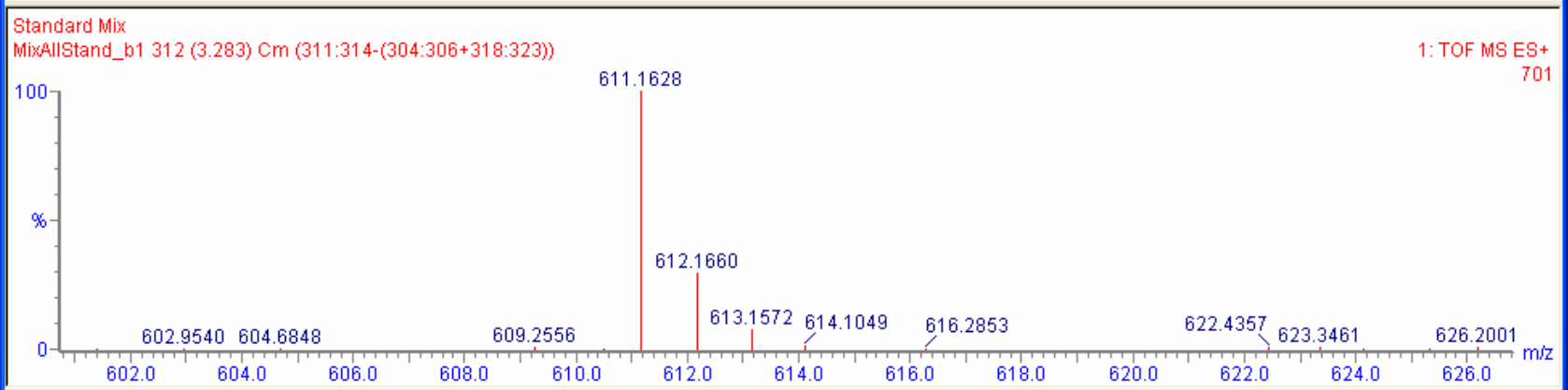
# Elemental composition analysis of 611.1628

Elemental Composition

File Edit View Process Help

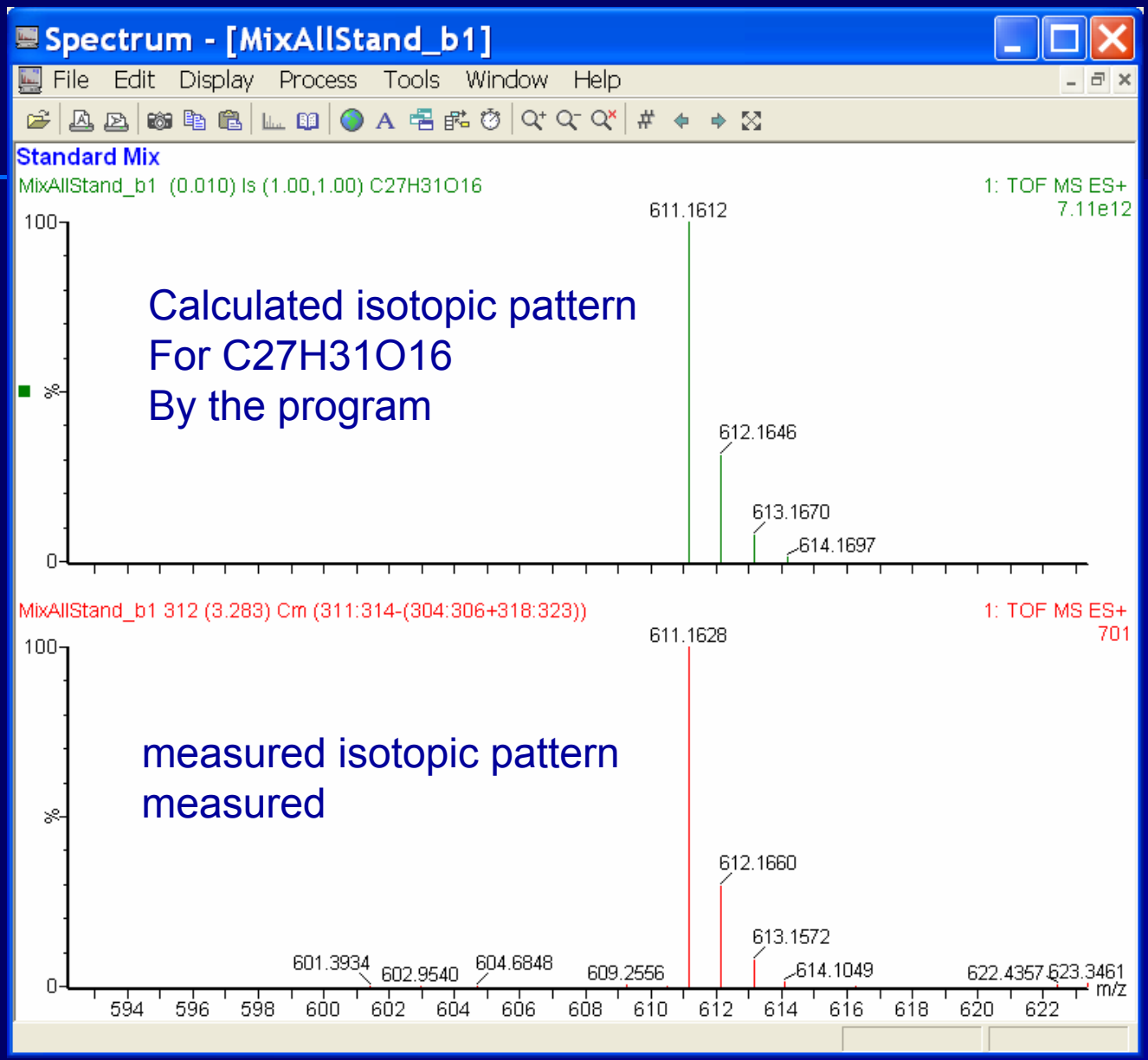
Single Mass Analysis (displaying only valid results)
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 50.0
Selected filters: None
**Monoisotopic Mass, Even Electron Ions**
**2415 formula(e) evaluated with 15 results within limits (up to 10 best isotopic matches for each mass)**

| Mass | Calc. Mass | mDa | PPM | DBE | Formula | i-FIT | C | H | N | O |
|---|---|---|---|---|---|---|---|---|---|---|
| 611.1628 | 611.1612 | 1.6 | 2.6 | 12.5 | C27 H31 O16 | 0.3 | 27 | 31 | | 16 |
| | 611.1599 | 2.9 | 4.7 | 18.5 | C24 H23 N10 O10 | 0.8 | 24 | 23 | 10 | 10 |
| | 611.1625 | 0.3 | 0.5 | 17.5 | C28 H27 N4 O12 | 1.4 | 28 | 27 | 4 | 12 |
| | 611.1612 | 1.6 | 2.6 | 23.5 | C25 H19 N14 O6 | 2.1 | 25 | 19 | 14 | 6 |
| | 611.1639 | -1.1 | -1.8 | 22.5 | C29 H23 N8 O8 | 3.5 | 29 | 23 | 8 | 8 |
| | 611.1625 | 0.3 | 0.5 | 28.5 | C26 H15 N18 O2 | 4.3 | 26 | 15 | 18 | 2 |
| | 611.1657 | -2.9 | -4.7 | 9.5 | C17 H27 N10 O15 | 5.0 | 17 | 27 | 10 | 15 |
| | 611.1652 | -2.4 | -3.9 | 27.5 | C30 H19 N12 O4 | 6.3 | 30 | 19 | 12 | 4 |
| | 611.1644 | -1.6 | -2.6 | 15.5 | C14 H19 N20 O9 | 7.6 | 14 | 19 | 20 | 9 |
| | 611.1644 | -1.6 | -2.6 | 4.5 | C16 H31 N6 O19 | 8.0 | 16 | 31 | 6 | 19 |




For Help, press F1

# Isotope pattern matching of 611.1628



See You Next Week