$^{13}C/^{12}C$ ratios for metabolite identification and quantitation

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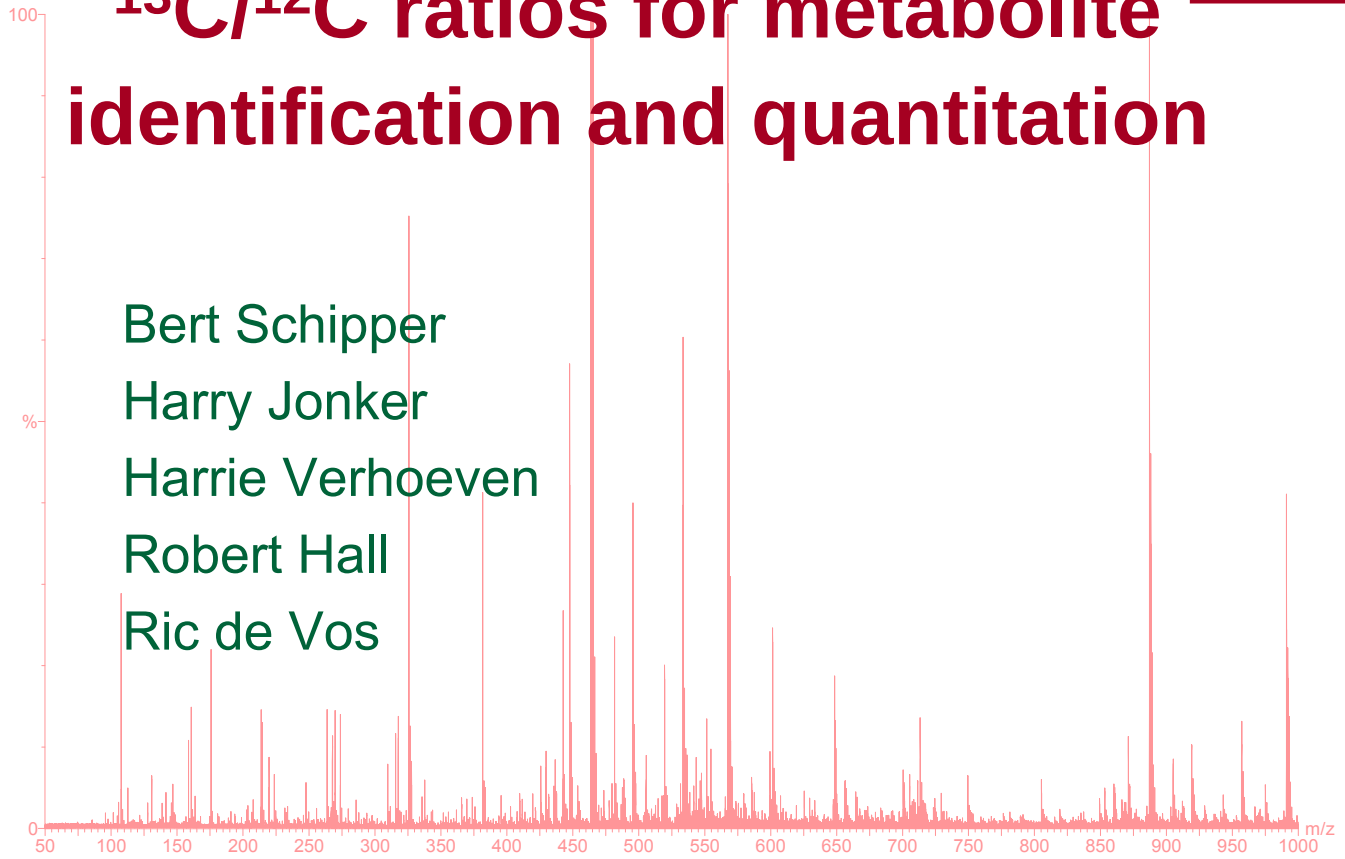


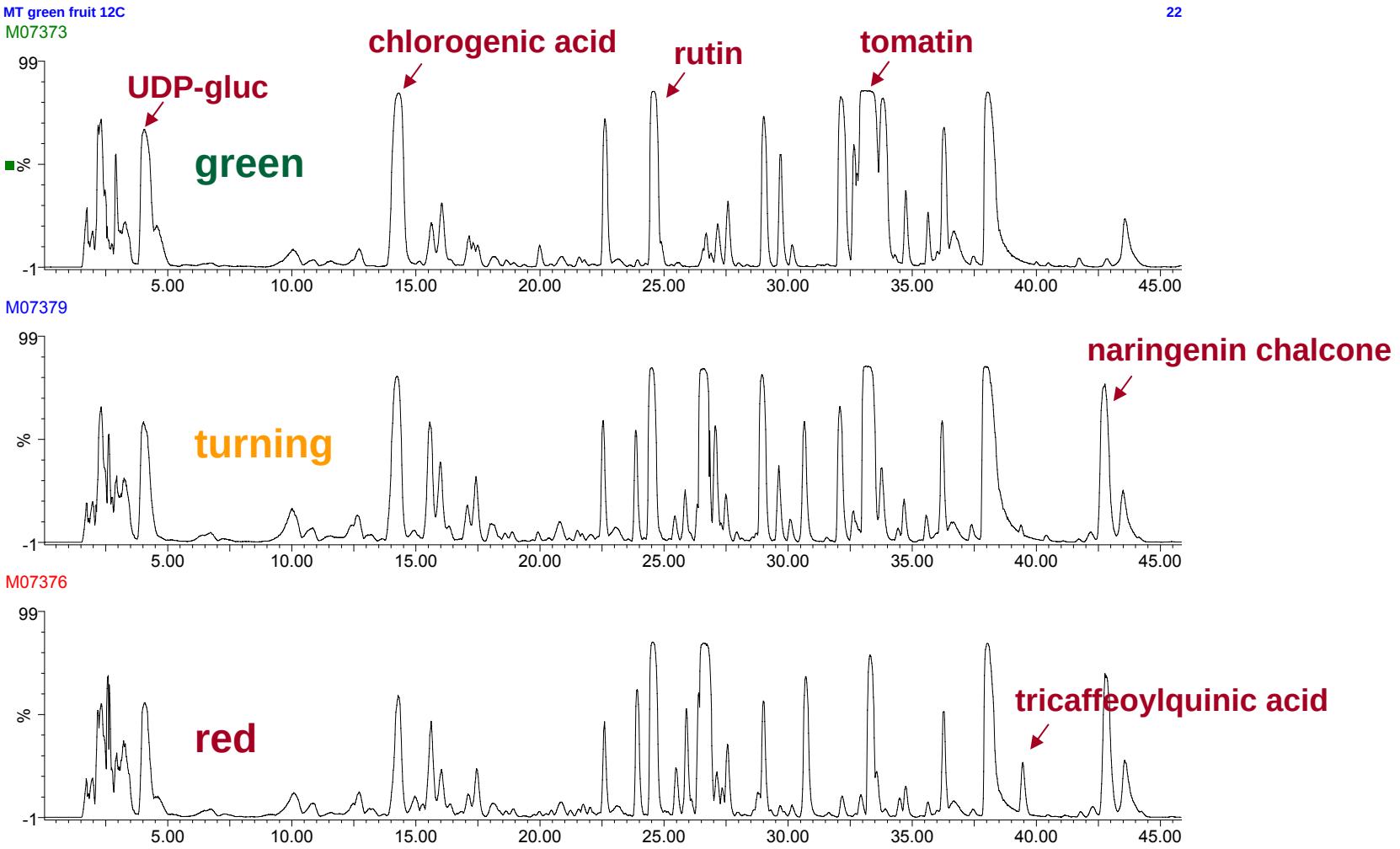
InD6-bottleneck project

- Novel and fundamental strategies for compound identification for generic metabolomics applications
- CBSG-grant, Aug. 2006 – Dec. 2007
- Aim:
  - Novel identification strategies
  - Generate core dataset of unambiguously identified metabolites
- Approaches:
  - Stable isotope-labelled plant material (*Iso*Life B.V.)
    - Improved elemental composition calculation
    - Additional: identifying matrix-dependent variation in extraction and detection of metabolites (GC-MS, LC-MS)
  - Database of reference compounds (retention time and MS/MS data)
    - development of models and tools for automated compound identification and annotation (i.c.w. Bioinformatics, group Roeland Van Ham)

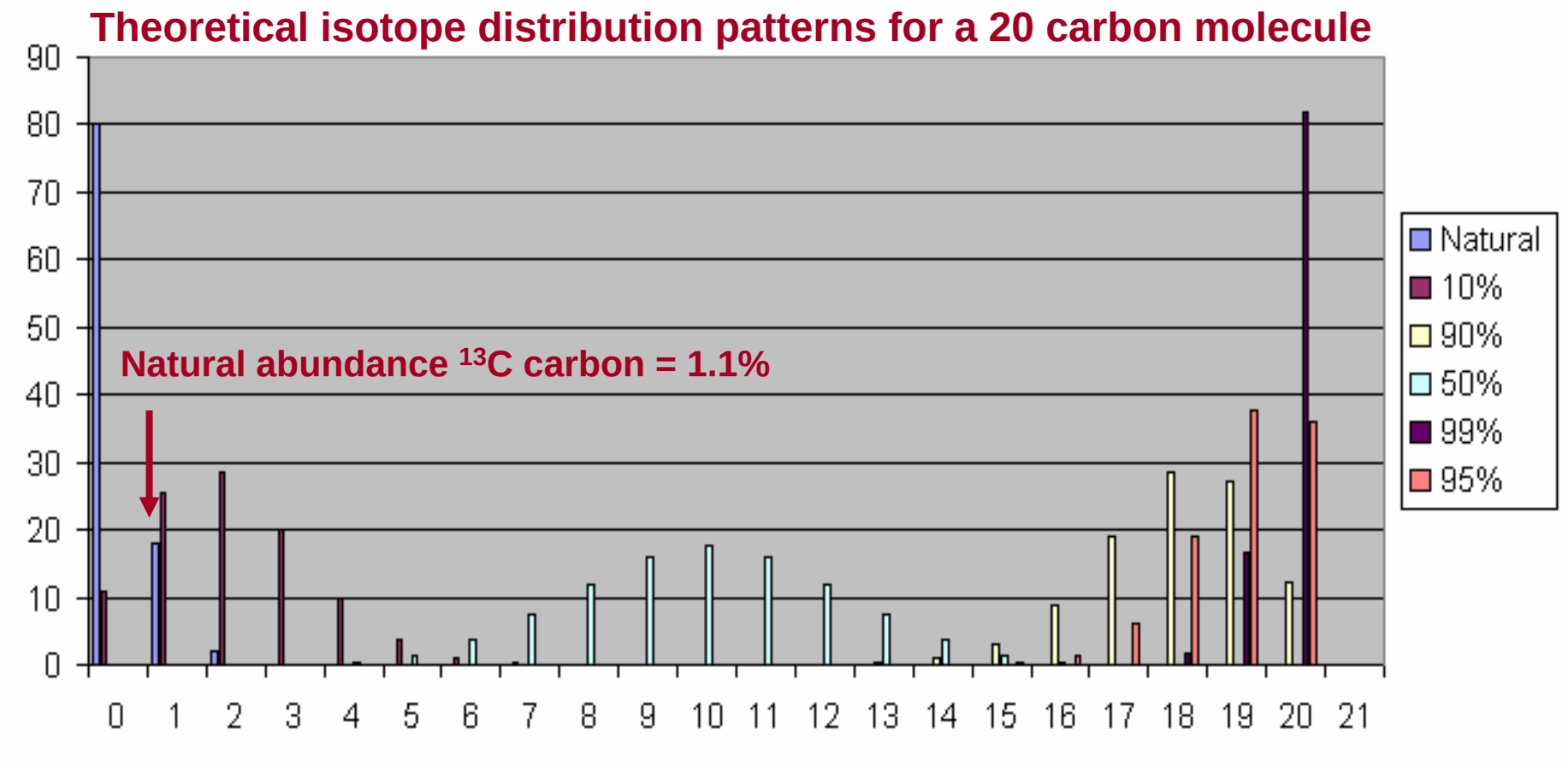
Stable isotope labelling of tomato: var. MicroTom

- *Iso*Life BV
  - Plants grown from seed to maturity air-tight ‘space capsules’, in either normal air ($^{12}CO_2$) or air with $^{13}CO_2$
  - ^{12}C- and ^{13}C-material (98 atom %) of mature plants harvested
    - fruits at green, turning and red stage of development
    - leaves
    - roots

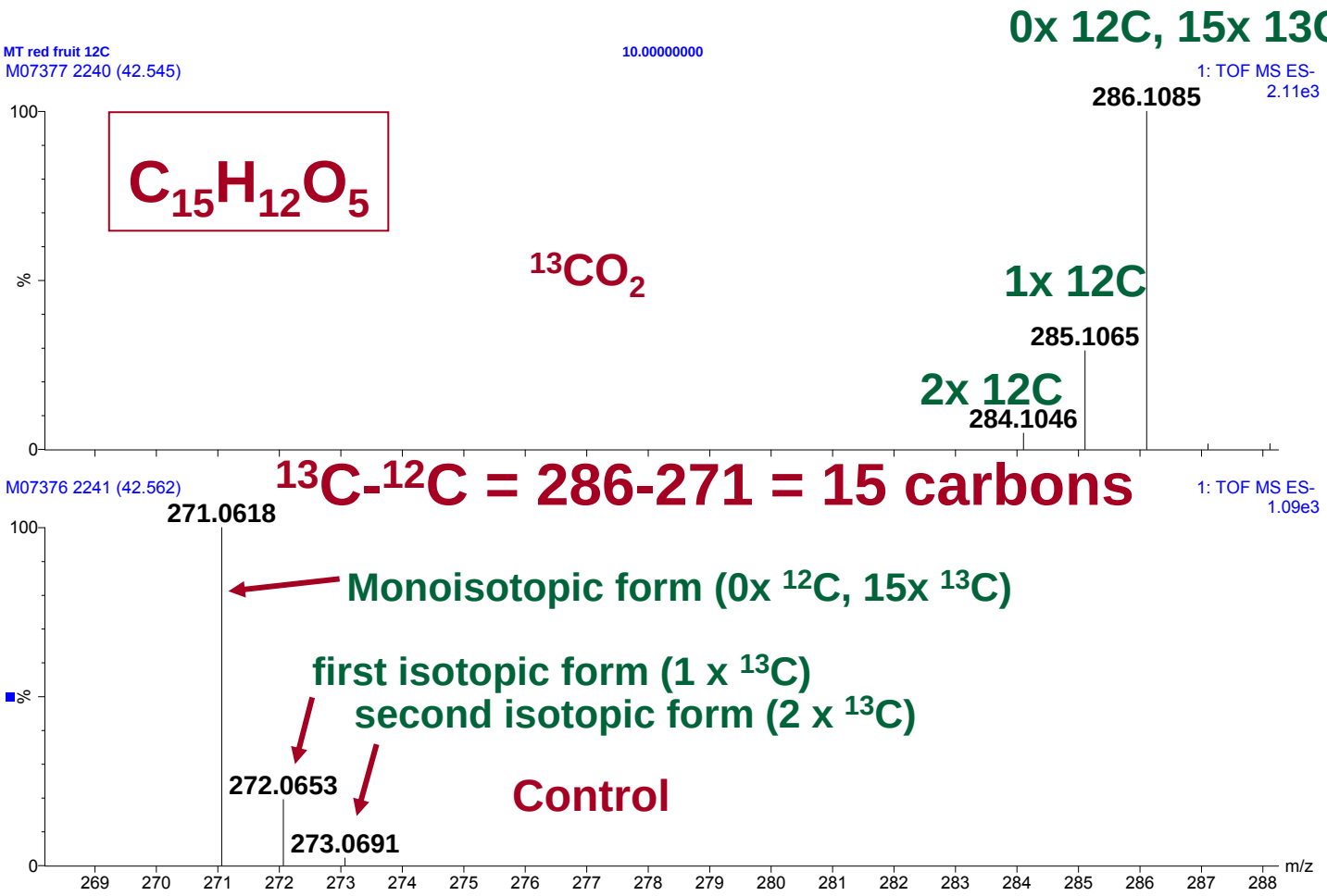
LCMS profiles of ripening MicroTom fruit



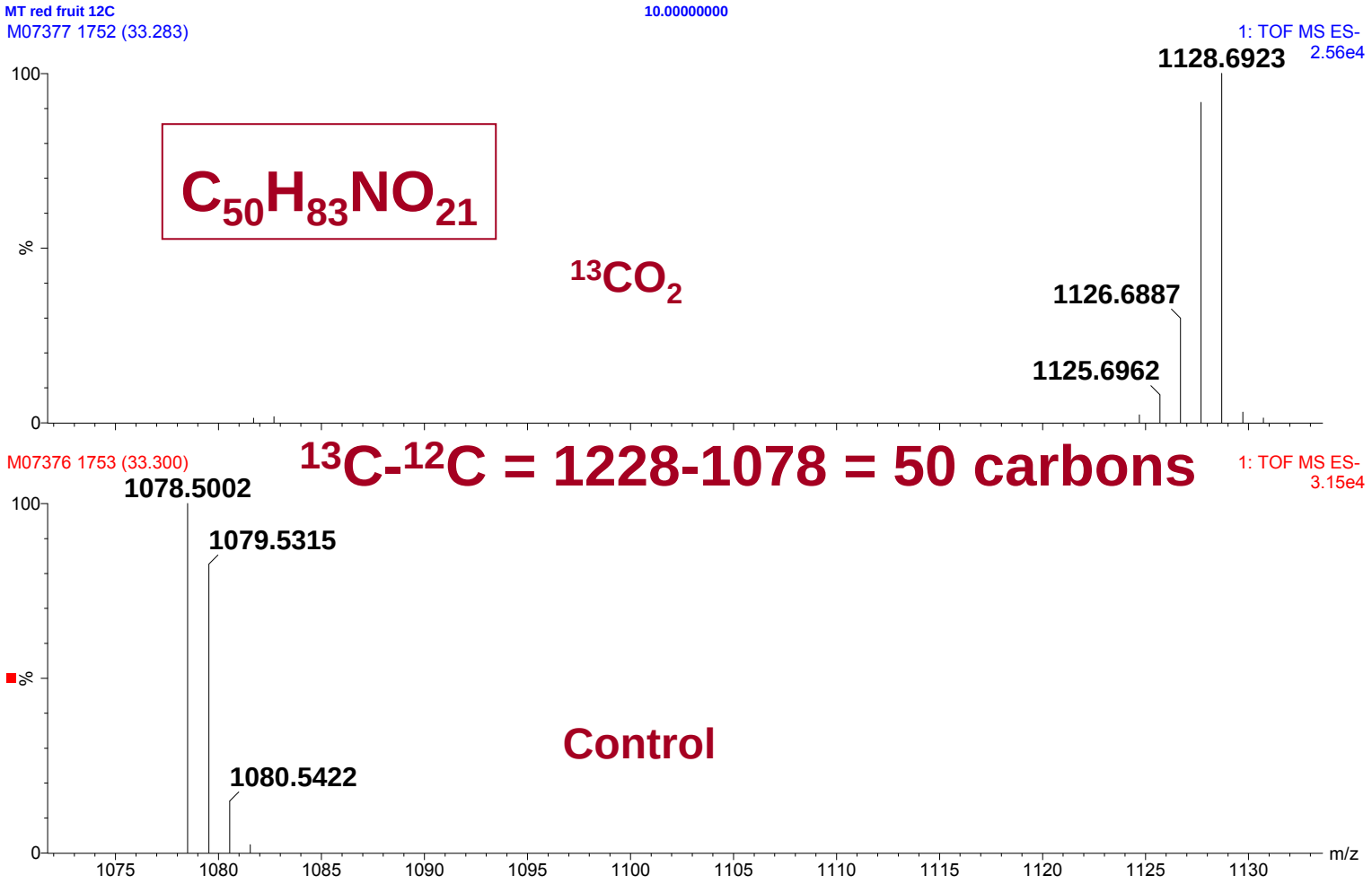
Effect of labeling efficiency on expected ^{13}C distribution



Isotopic forms of some known tomato fruit metabolites: naringenin-chalcone



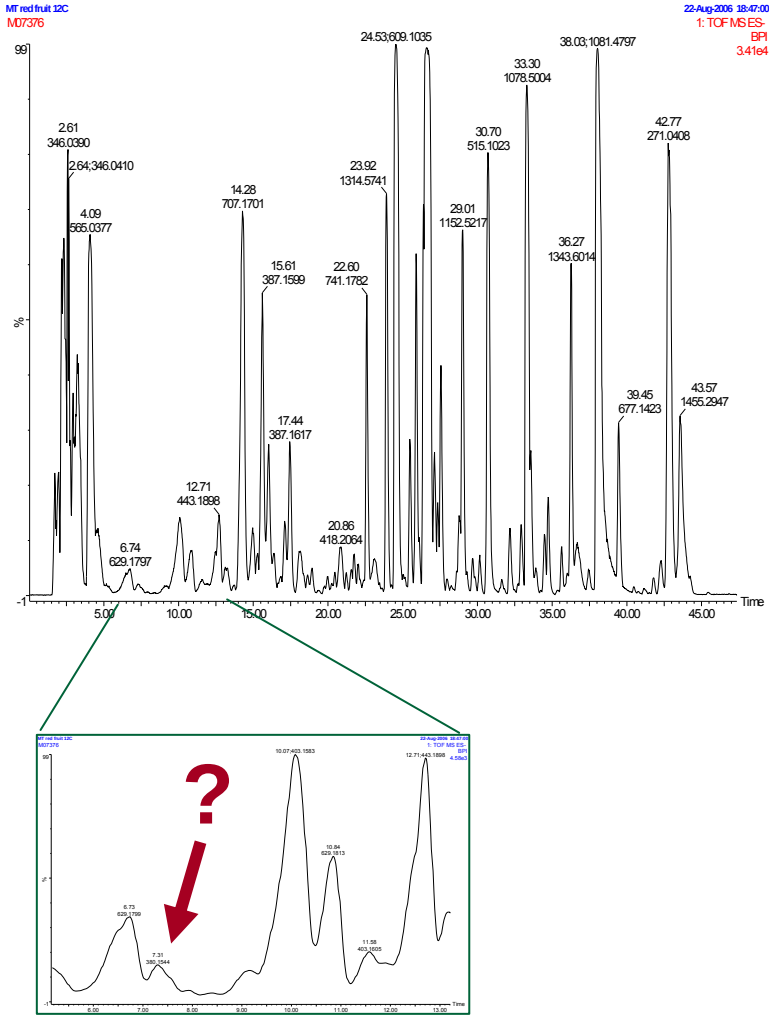
Isotopic forms of α-tomatin



Tomato ^{13}C-labelling efficiency

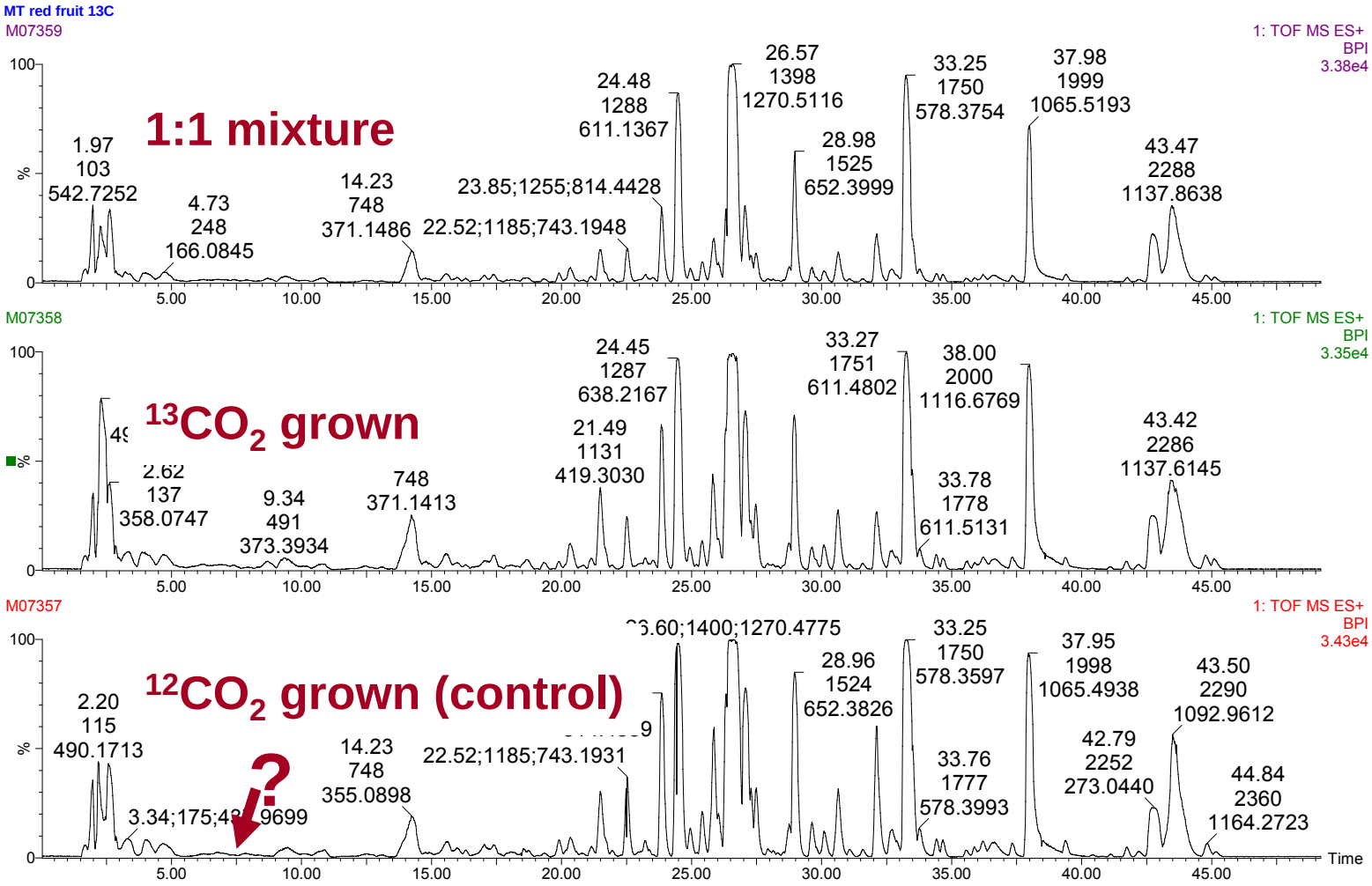
- Based on isotopic distribution of known compounds: ^{13}C-labelling efficiency of all metabolites: > 98 atom %
- Inverted isotopic distribution in ^{13}C-labelled metabolites in range from 1 to 56 carbon atoms
  - At higher carbon numbers: fully-labeled ^{13}C mass is not the highest signal (spectra more difficult to interpret)

Use of ^{13}C in compound identification

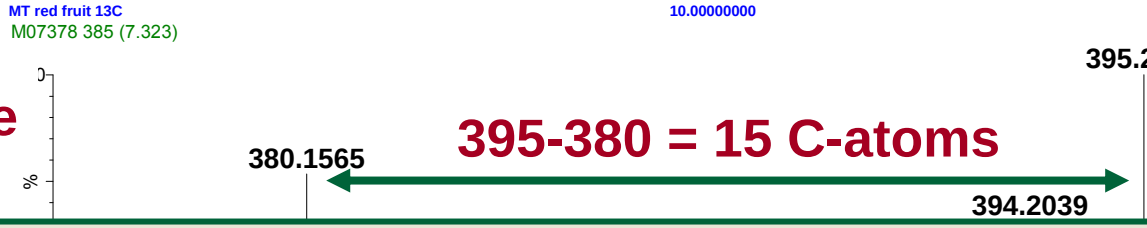


- most abundant peaks detected in aqueous-methanol extracts have been (putatively) identified
  - accurate mass, MS/MS, PDA
  - MoTo-DB (i.c.w. InD5)
    - mainly secondary metabolites
- small <u>unknown</u> peaks: difficult to define an unequivocal elemental composition
  - increase mass accuracy (<1 ppm)
    - resolution > 60.000 (FTMS)
  - determine exact number of carbons
    - ^{13}C-stable isotope labeling

^{13}C-labelling to determine exact number of carbon atoms in molecule



Mass spectra of ^{13}C-labelled and control tomato fruits, at rt = 7.3 min



Mass	Calc. Mass	mDa	PPM	Formula	C	H	N	O	P	S
380.1560	380.1562	-0.2	-0.4	C18 H27 N3 O2 P S	18	27	3	2	1	1
	380.1557	0.3	0.9	C15 H26 N O10	15	26	1	10		
	380.1555	0.5	1.3	C10 H31 N5 O4 P S2	10	31	5	4	1	2
	380.1565	-0.5	-1.4	C16 H30 N O5 S2	16	30	1	5		2
	380.1568	-0.8	-2.1	C10 H33 N5 O2 P3 S	10	33	5	2	3	1
	380.1568	-0.8	-2.1	C26 H23 N P	26	23	1		1	
	380.1551	0.9	2.4	C11 H34 N3 O3 P4	11	34	3	3	4	
	380.1570	-1.0	-2.7	C16 H22 N5 O6	16	22	5	6		
	380.1546	1.4	3.6	C9 H27 N5 O9 P	9	27	5	9	1	
	380.1574	-1.4	-3.8	C18 H29 N3 P3	18	29	3		3	
	380.1545	1.5	3.9	C20 H22 N5 O S	20	22	5	1		1
	380.1544	1.6	4.1	C19 H28 N O3 P2	19	28	1	3	2	
	380.1578	-1.8	-4.8	C16 H32 N O3 P2 S	16	32	1	3	2	1
	380.1579	-1.9	-4.9	C17 H26 N5 O S2	17	26	5	1		2

At 5 ppm accuracy, 15 C atoms: only 1 chemical formula possible

Further use of ^{13}C-labelled material in plant metabolomics

- (^{12}C) samples with and without same amount of ^{13}C material added at start of extraction
- Determine ratio of ^{12}C vs ^{13}C metabolite:
  - ^{13}C as internal standard for all common metabolites
    - Correction for matrix-dependent differences in extraction efficiency, recovery, ionisation, etc. (6-fold reduction in CV%)
  - Allows to correlate different experiments
    - over time, tissues etc, by correction for analytical variation in ^{13}C peaks
  - Allows quantification *a posteriori*
- So far: 155 extracts prepared and analyzed
  - Concentration range of both ^{12}C and ^{13}C red fruit, with and without concentration range of internal standards
  - ^{12}C and ^{13}C red fruit mixed in different ratios
  - All in three replicates

Collaboration

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- Joost de Groot
- Egon Willighagen
- Roeland van Ham
- Ton Gorissen
- Ries de Visser