

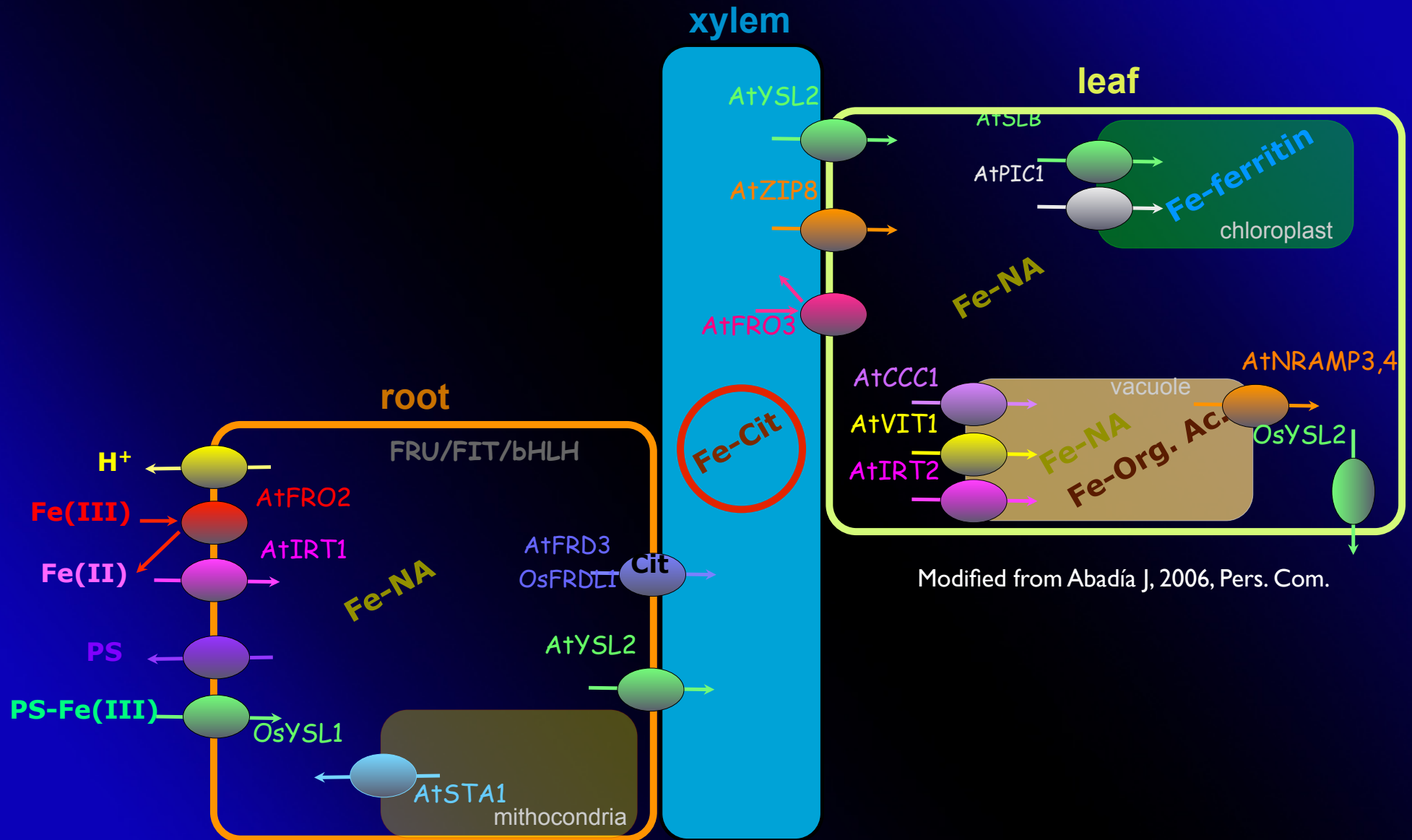
Identificación de complejos organometálicos en tejidos vegetales mediante espectrometría de masas

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www.eead.csic.es/stressphysiology



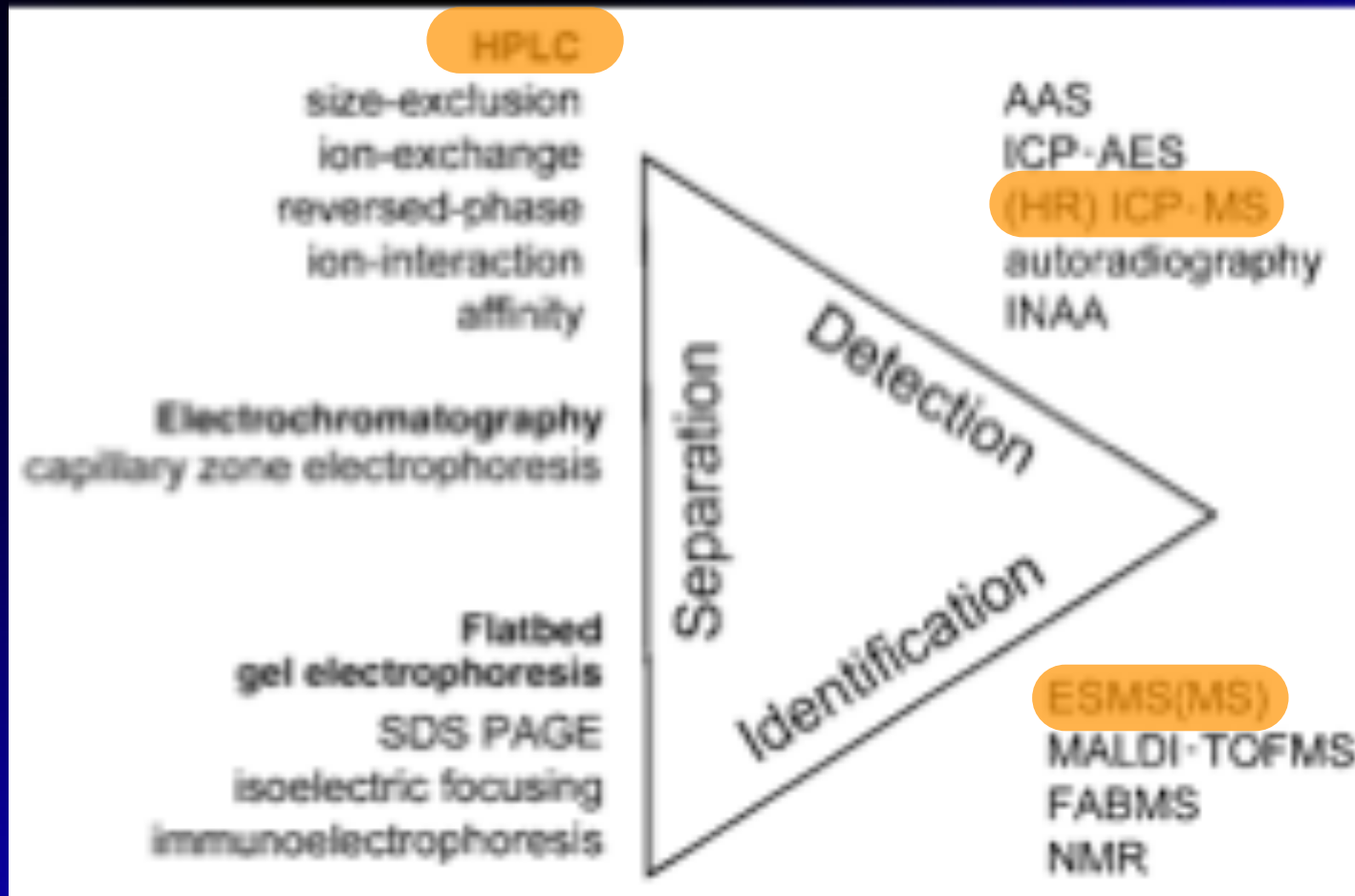
Metal Speciation: A complex(ed) story



Modified from Abadía J, 2006, Pers. Com.



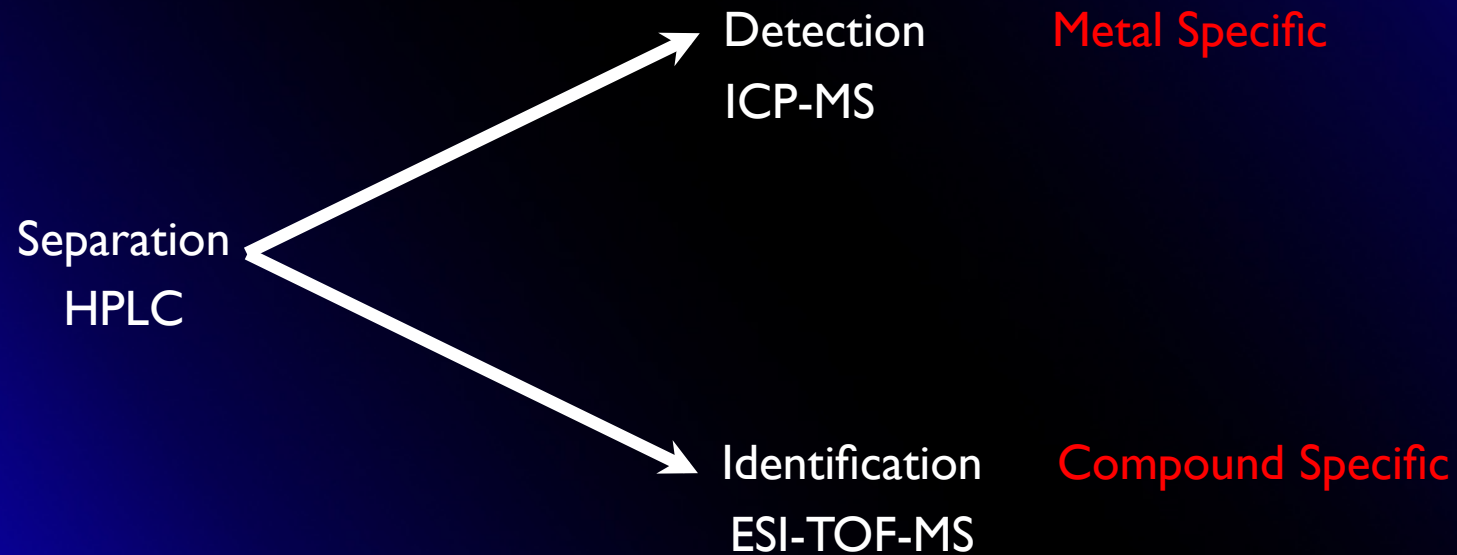
Hyphenated techniques available for species-selective analysis of biological materials



Szpunar, 2000



Hyphenated techniques available for species-selective analysis of biological materials



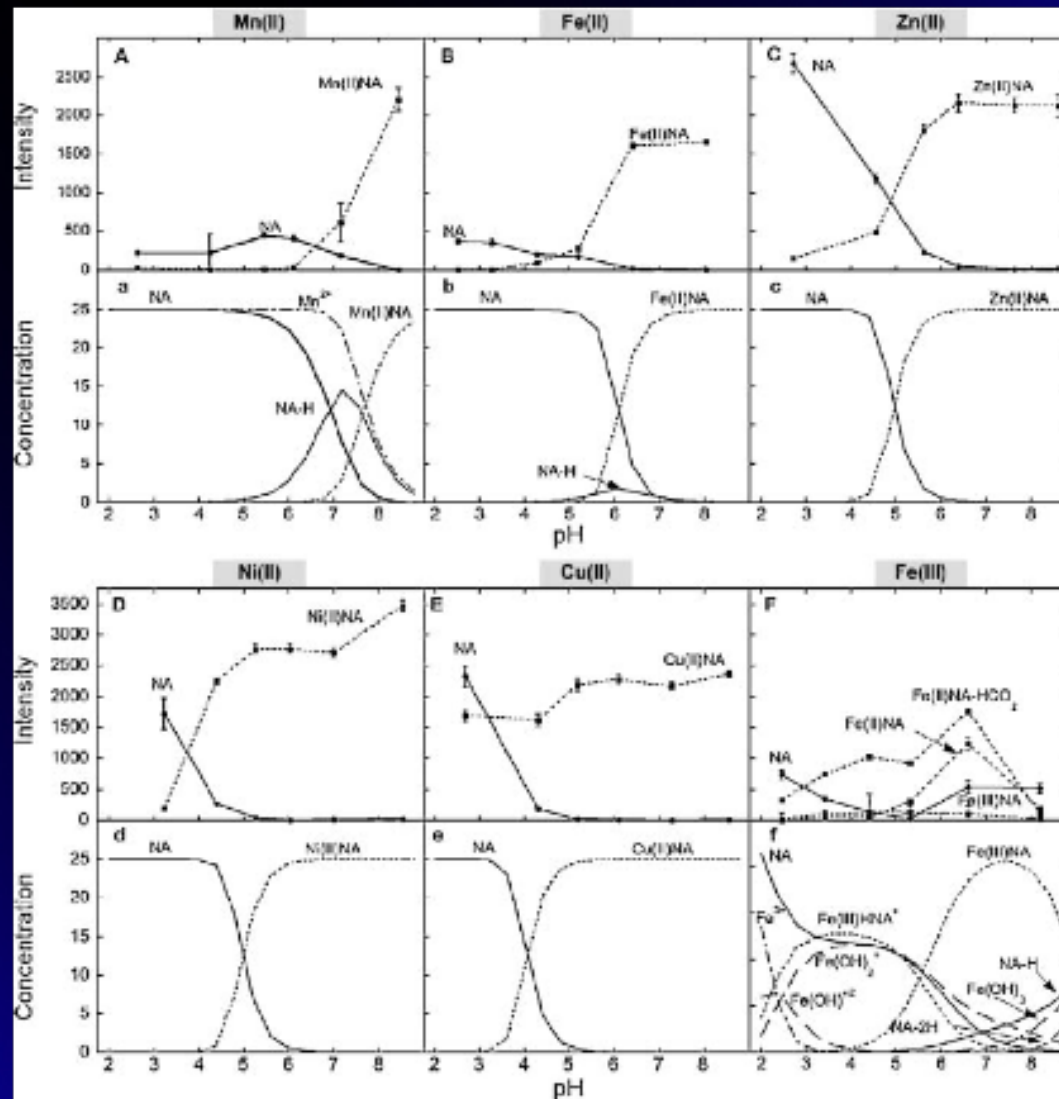
Main problems and bottlenecks

-Sample extraction: should conserve the plant compartment conditions for example pH values.



Main problems and bottlenecks

pH Dependence

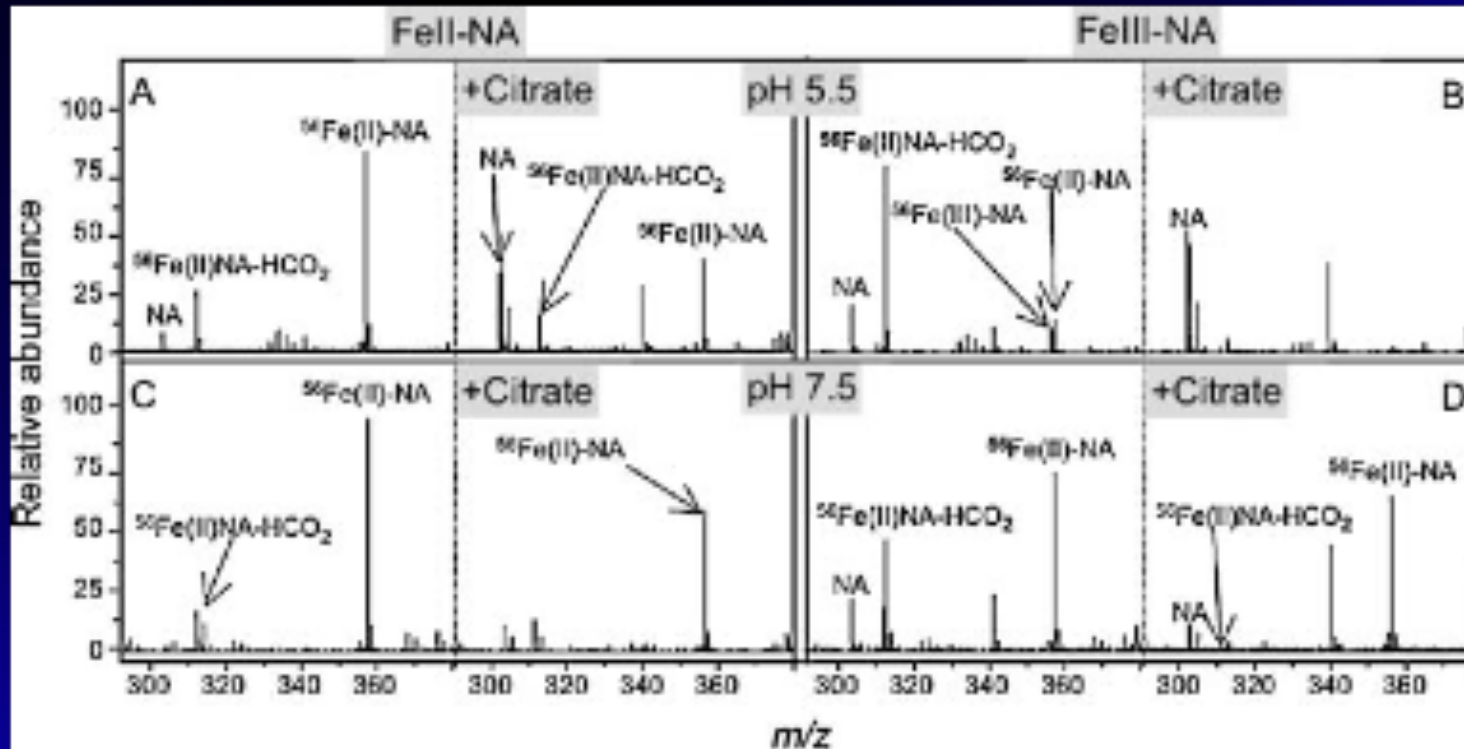


Rellan et al, 2008



Main problems and bottlenecks

pH Dependence
Ligand Exchange



Rellan et al, 2008



Main problems and bottlenecks

- Sample extraction: should conserve the plant compartment conditions for example pH values.
- These conditions should also be conserved during separation and detection.
- If this conditions a
- Sensitivity: very low concentration of metals in plant tissues.
- Cost: expensive equipment.

Integrated mass spectrometry in (semi-)metal speciation

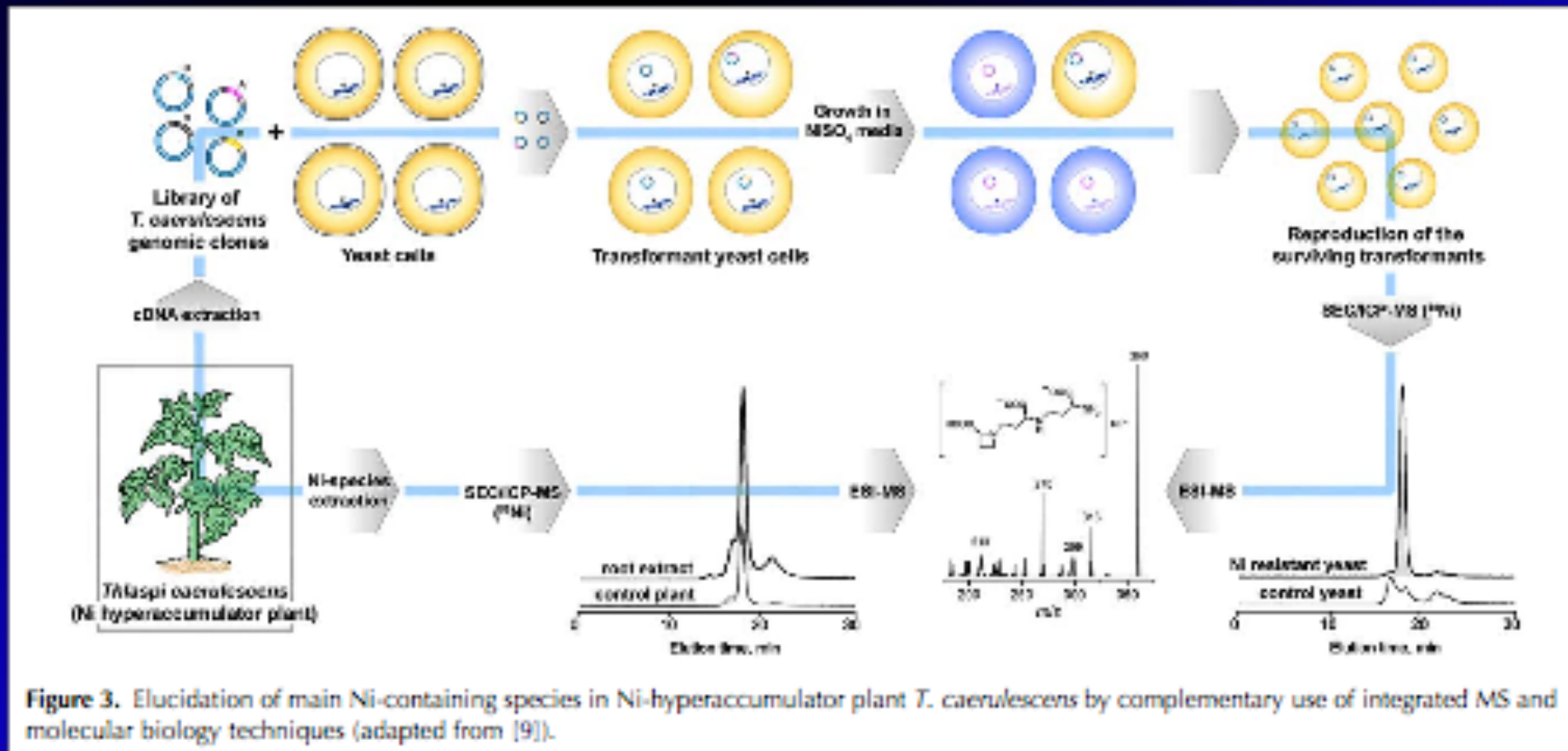


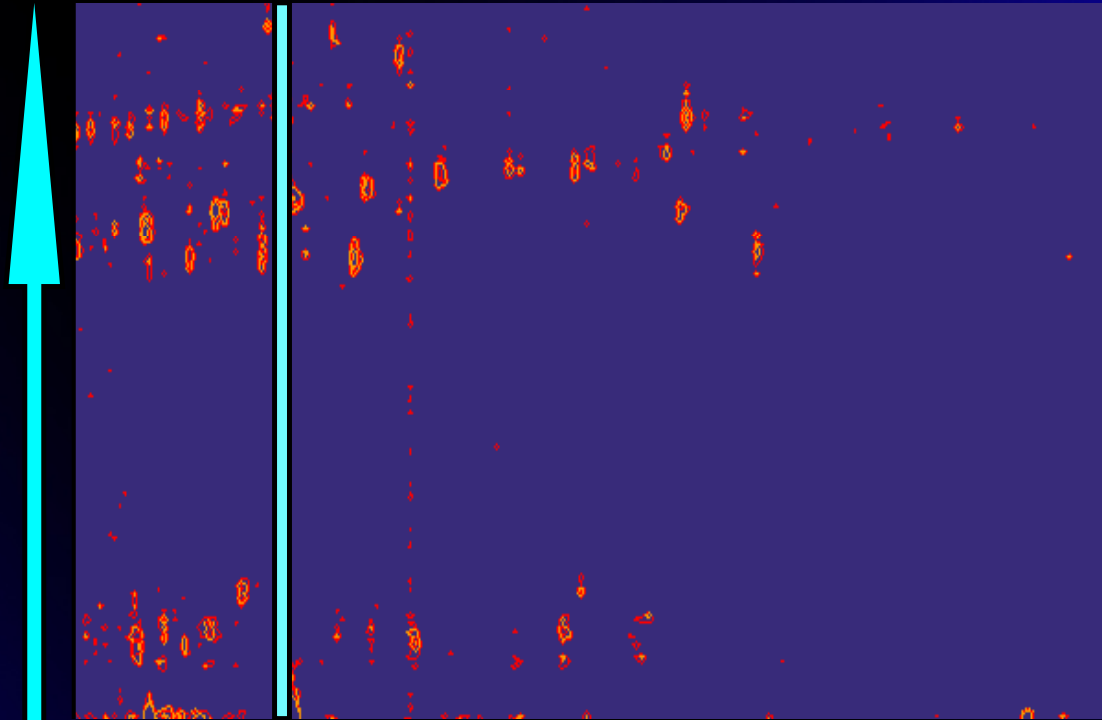
Figure 3. Elucidation of main Ni-containing species in Ni-hyperaccumulator plant *T. caerulescens* by complementary use of integrated MS and molecular biology techniques (adapted from [9]).

Meija, 2006



HPLC-TOFMS

m/z
TOFMS

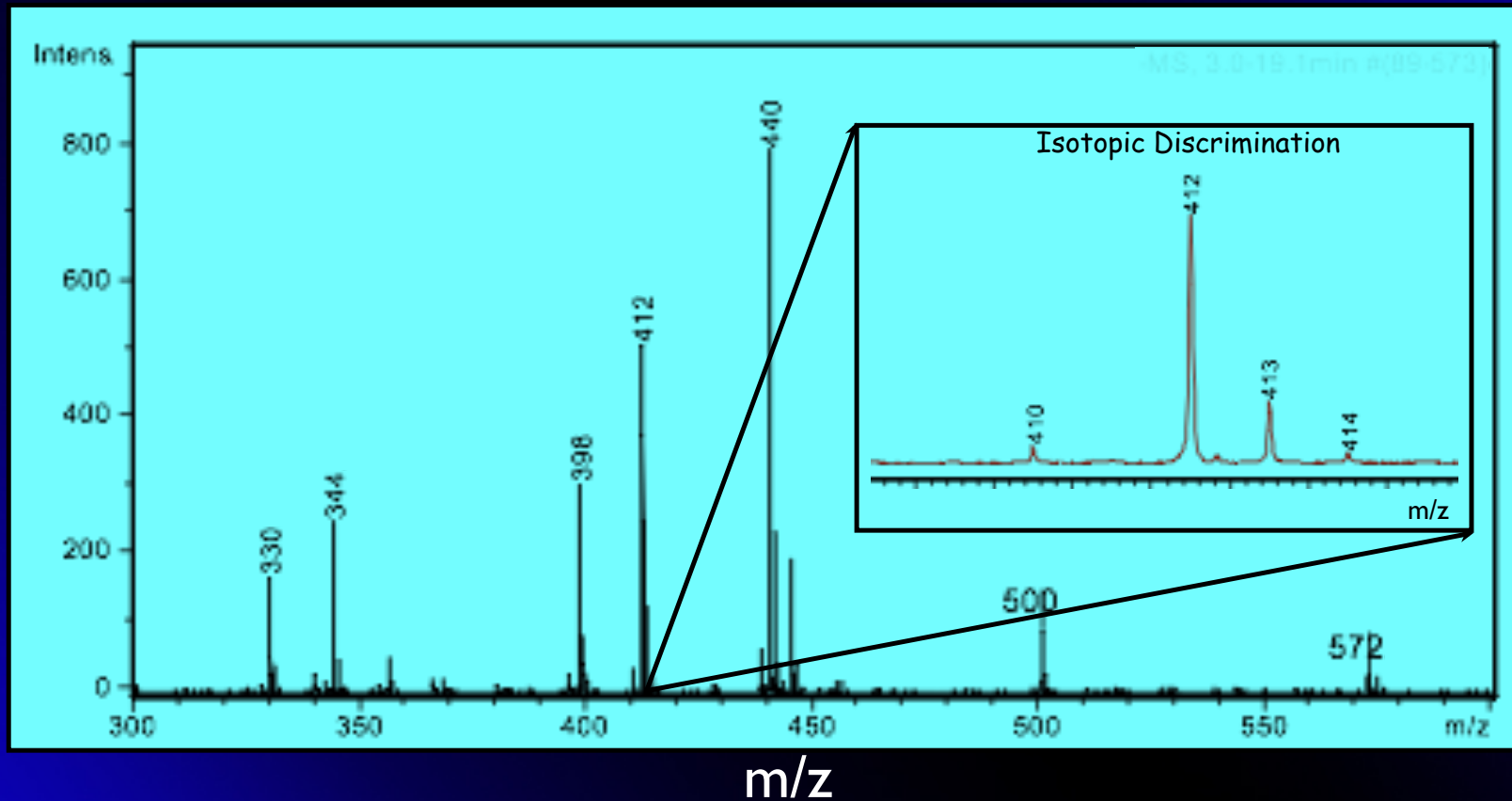


time (HPLC)



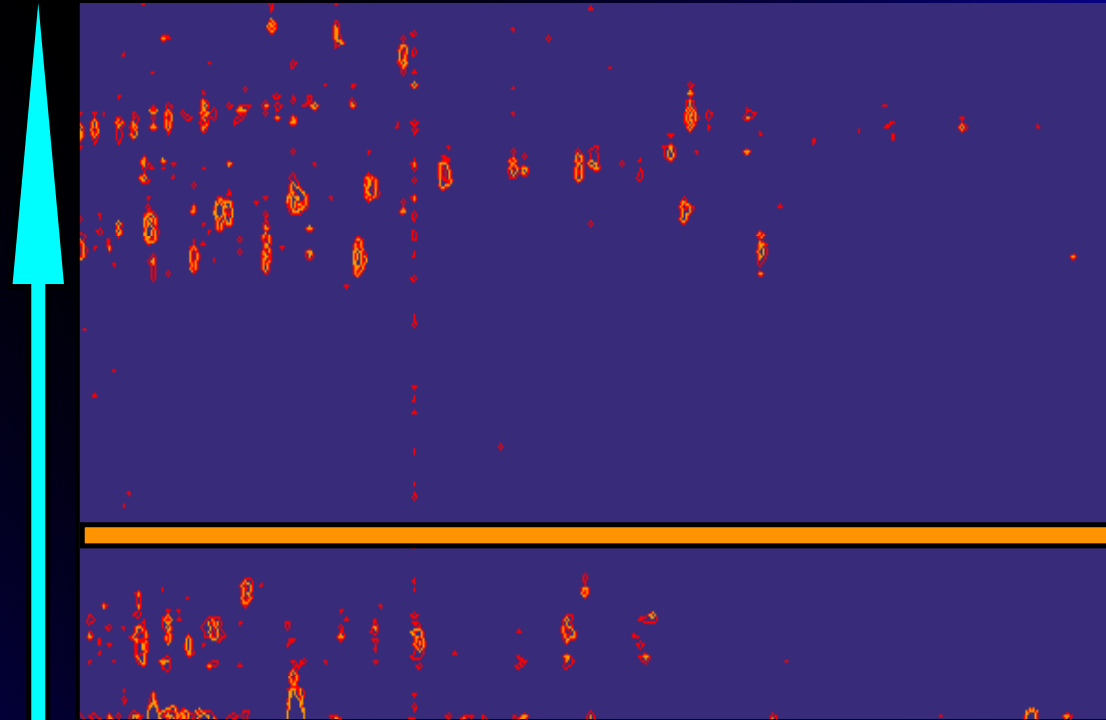
HPLC-TOFMS Mass spectra at an specific time

5.9 min



HPLC-TOFMS

m/z
TOFMS

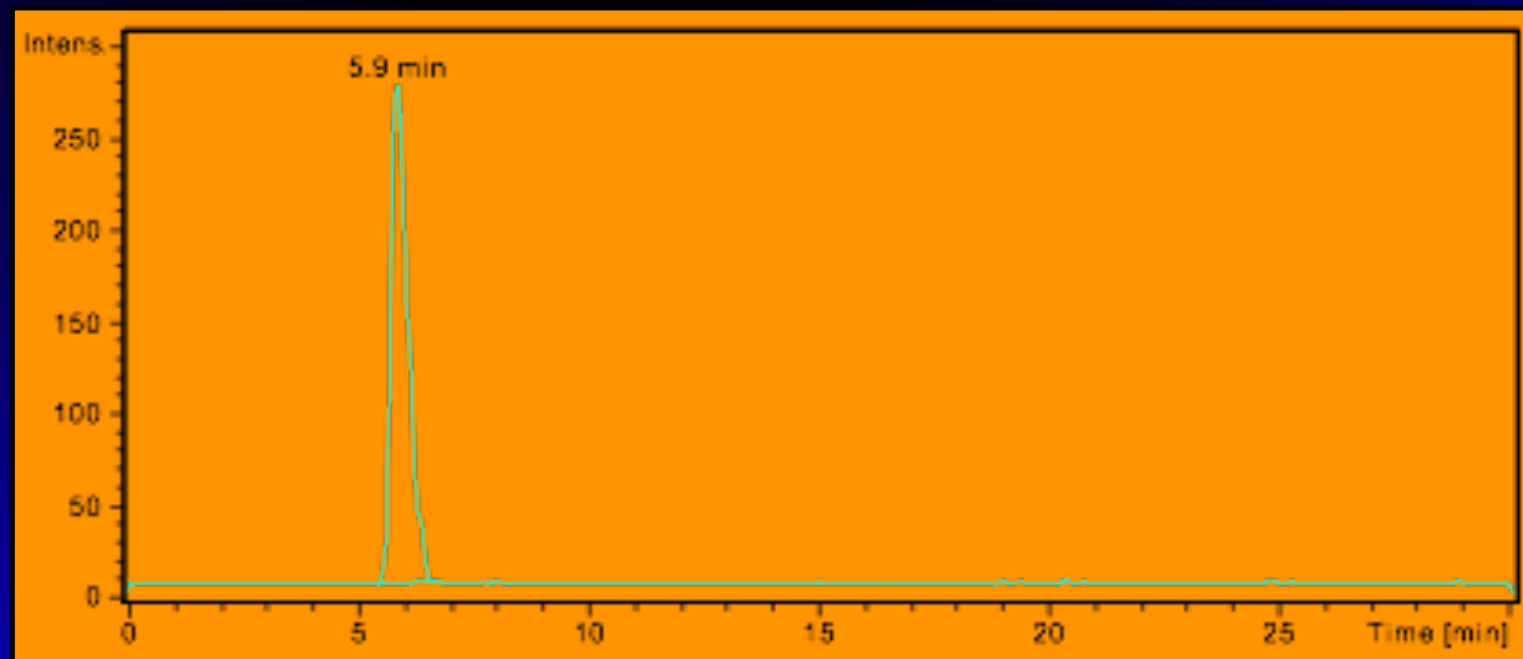


time (HPLC)



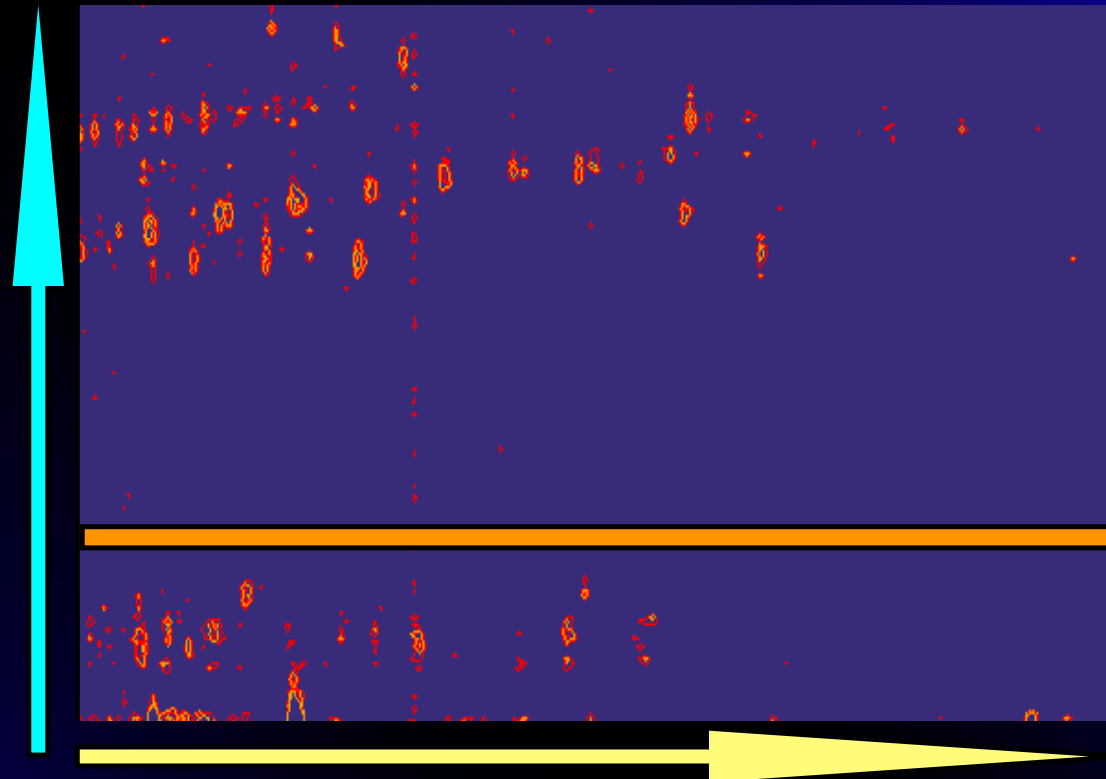
HPLC-TOFMS Chromatogram of an specific m/z

$m/z = 344$



HPLC-TOFMS

m/z
TOFMS

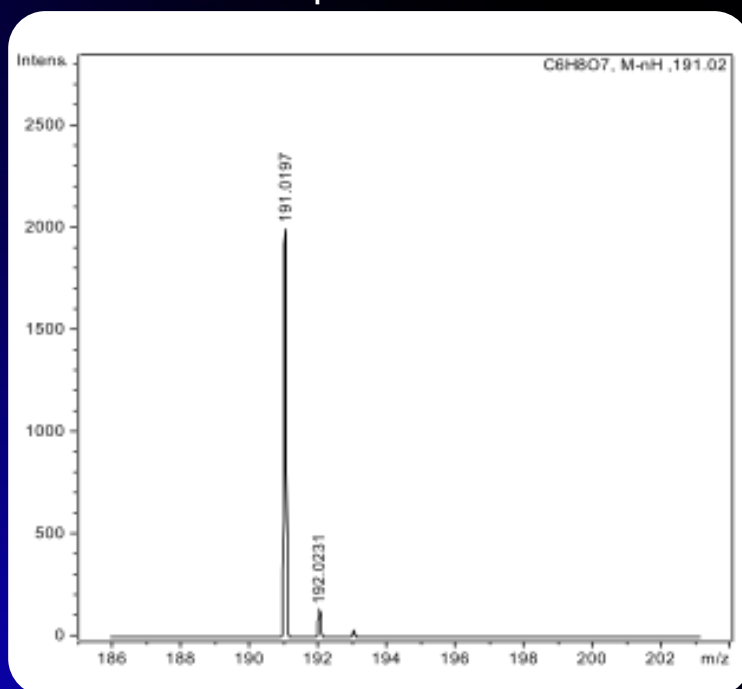


time (HPLC)

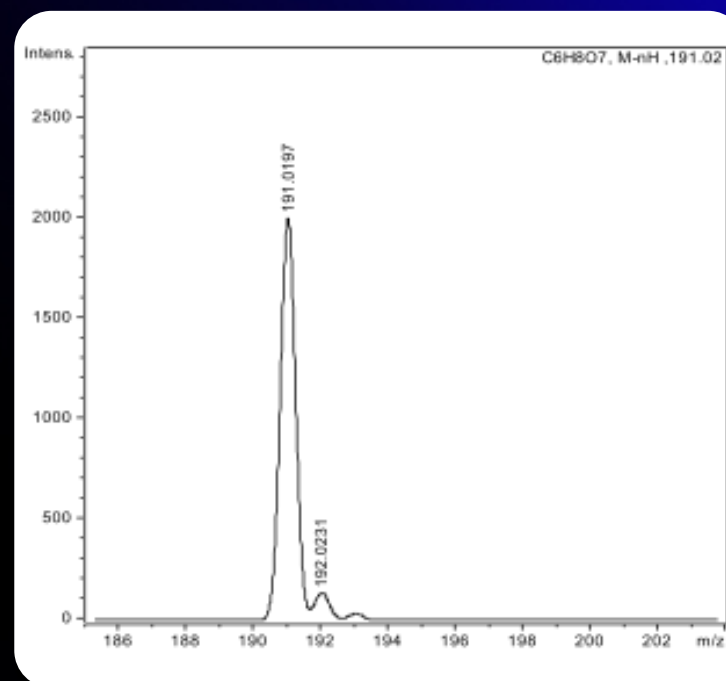


Compound identification based on isotopic signature

Good Isotopic Discrimination

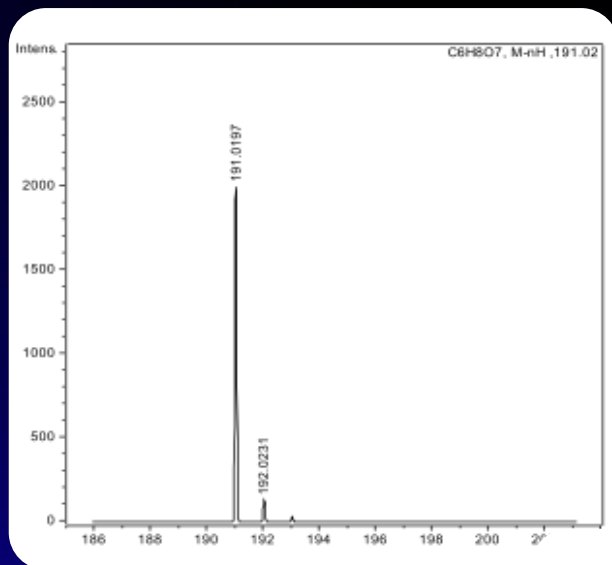


Bad Isotopic Discrimination

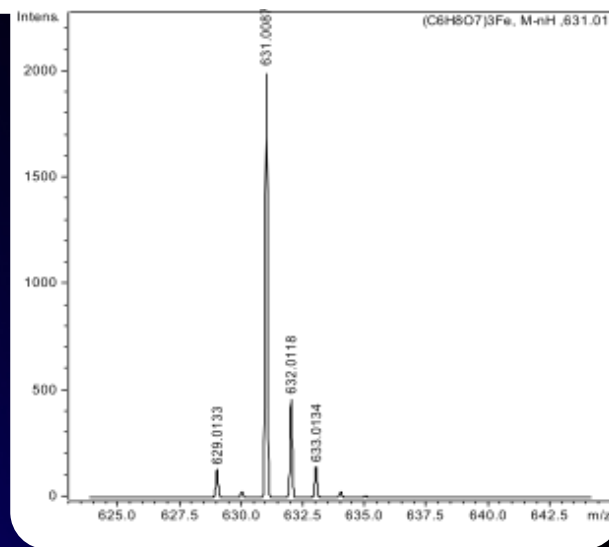
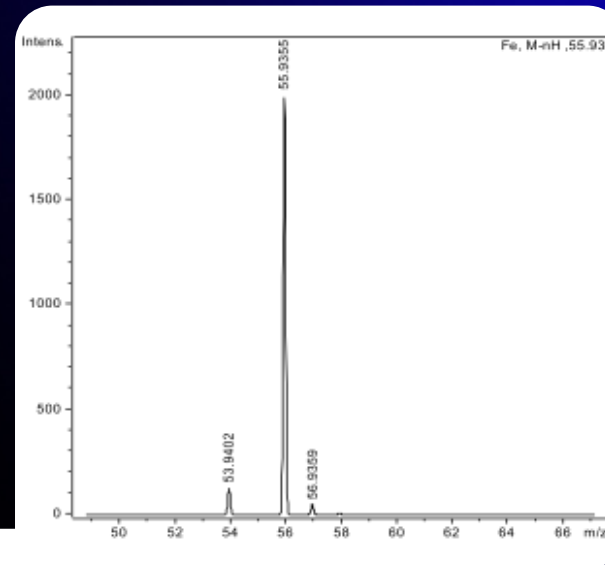


Compound identification based on isotopic signature

CHO Isotopic Distribution

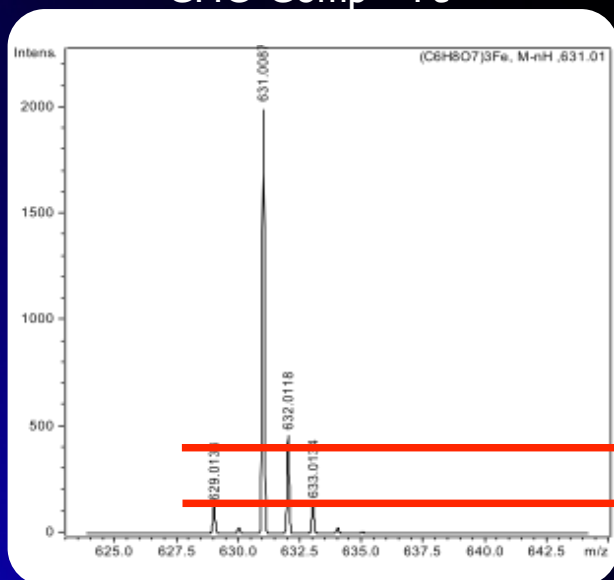


Fe Isotopic Distribution

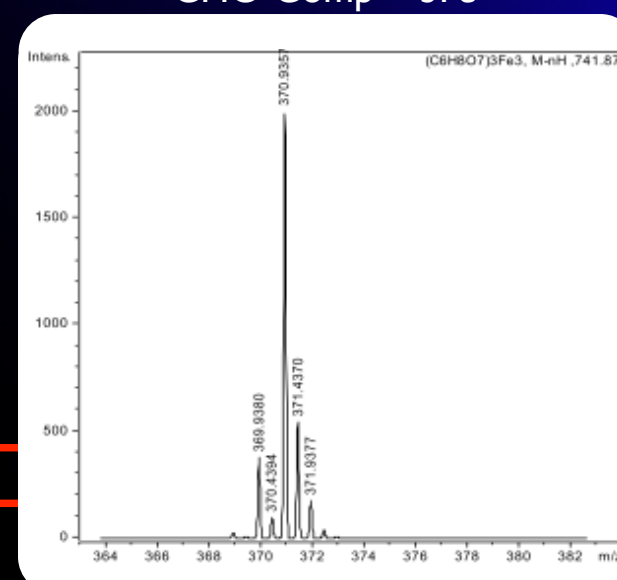


Compound identification based on isotopic signature

CHO Comp + Fe



CHO Comp + 3Fe



Compound identification based on isotopic signature

<http://www.biomedcentral.com/1471-2105/7/234>

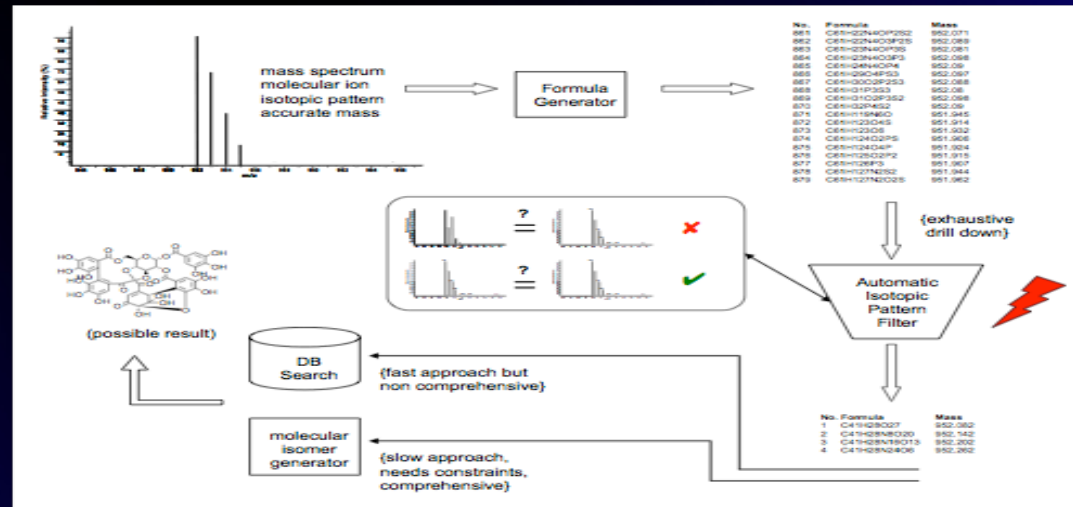


Figure 1. A flowchart illustrating a compound identification process based on mass spectrometric calculation of elemental compositions and subsequent database searches. The process starts with a mass spectrum (m/z vs. relative intensity) and a molecular ion isotopic pattern. This leads to a 'Formula Generator' which produces a list of possible formulas and their masses. An 'Automatic Isotopic Pattern Filter' then compares the experimental isotopic pattern against these candidates, using an 'exhaustive drill down' process. A 'DB Search' (fast approach but non-comprehensive) and a 'molecular isomer generator' (slow approach, needs constraints, comprehensive) also feed into the filter. The final output is a list of four possible formulas: C4H8O2, C4H8NO2, C4H8N2O2, and C4H8N2O6.

types in metabolites which can be used to identify the source of the metabolite. The isotopic signature of a metabolite's mass spectrum can be used as a constraint for removing some of the possible candidates. Isotope ratio mass spectrometry can exactly determine isotope abundances, but is only applicable to combustions of the original metabolite into gases and therefore irrelevant for the calculation of elemental compositions of unidentified metabolites. In general, the theoretical isotope abundances of a metabolite's molecular formula can be calculated using either fast approaches [12] either solving polynomial equations using fast Fourier transformations [13]. An isotopic abundance filter can be used for any mass spectrum and can provide very low root mean square (RMS) errors for isotopic patterns, especially if the contribution of isotopes

Kind and Fiehn, 2006

Compound identification based on isotopic signature

Mass accuracy vs isotopic abundance accuracy

BMC Bioinformatics 2006, 7:234

<http://www.biomedcentral.com/1471-2105/7/234>

Table 3: Number of possible molecular formulas at different levels of mass accuracy and the impact of isotopic abundance accuracy. A mass spectrometer capable of 3 ppm but with 2% correct isotopic pattern outperforms even a (non-existing) mass spectrometer with 0.1 ppm mass accuracy! The results are computed for randomly selected targets, so single results vary but the trend remains. LEWIS and SENIOR check was applied. Candidates with unrelated high element counts were already excluded

molecular mass [Da]	without isotope abundance information					2% isotopic abundance accuracy	5% isotopic abundance accuracy
	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	2	6
400	78	37	23	7	1	2	13
500	266	115	64	21	2	3	33
600	505	237	155	50	5	4	36
700	1046	338	321	108	10	10	97
800	1964	973	599	260	20	13	111
900	3447	1712	1045	343	32	18	196

implemented into query algorithms and are hard to conceive even at the 1 ppm level, when hundreds of possible

hits are returned at searches between 700–900 Da, the mass range of membrane lipids. The principal idea of

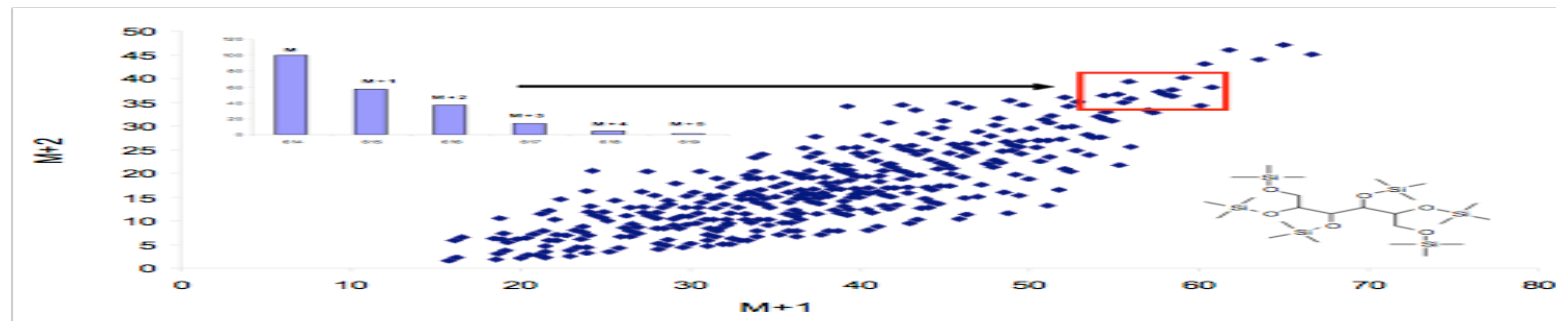


Figure 5 The isotopic abundances of the $M+1$ and $M+2$ ions can be used to filter molecular formula candidates. This example shows isotopic abundance pattern for silylated sorbitol. The red circle shows a 5% region with the correct target. All other formulae can be excluded if the mass spectrometer has a 5% error (RMS) on isotopic abundances.

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(page number not for citation purposes)

Kind and Fiehn, 2006



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Compound identification based on isotopic signature

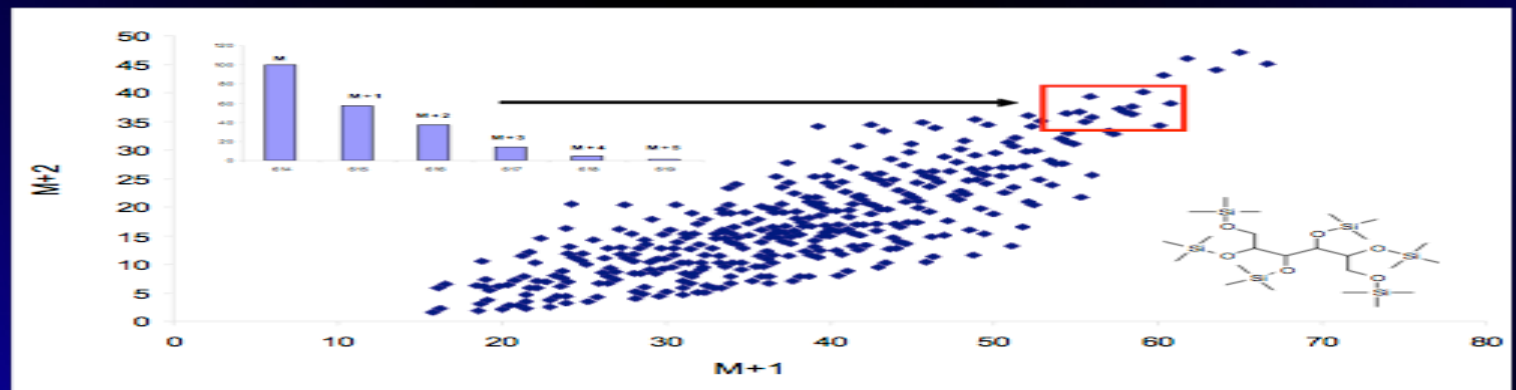
2006, Kind et al.

<http://www.biomedcentral.com/1471-2105/7/234>

Table 2. Accuracy of mass spectrometry at 0.1 ppm mass accuracy and 0.1 ppm mass accuracy and 0.1 ppm mass accuracy

molecular mass (Da)	0.1 ppm	0.1 ppm		3% isotopic abundance accuracy		5% isotopic abundance accuracy	
		0.1 ppm	3 ppm	3 ppm	5 ppm		
100	1	1	1	1	1	1	1
200	1	1	1	1	1	1	1
300	1	1	1	1	1	1	1
400	1	1	1	1	1	1	1
500	1	1	1	1	1	1	1
600	1	1	1	1	1	1	1
700	1	1	1	1	1	1	1
800	1	1	1	1	1	1	1
900	1	1	1	1	1	1	1

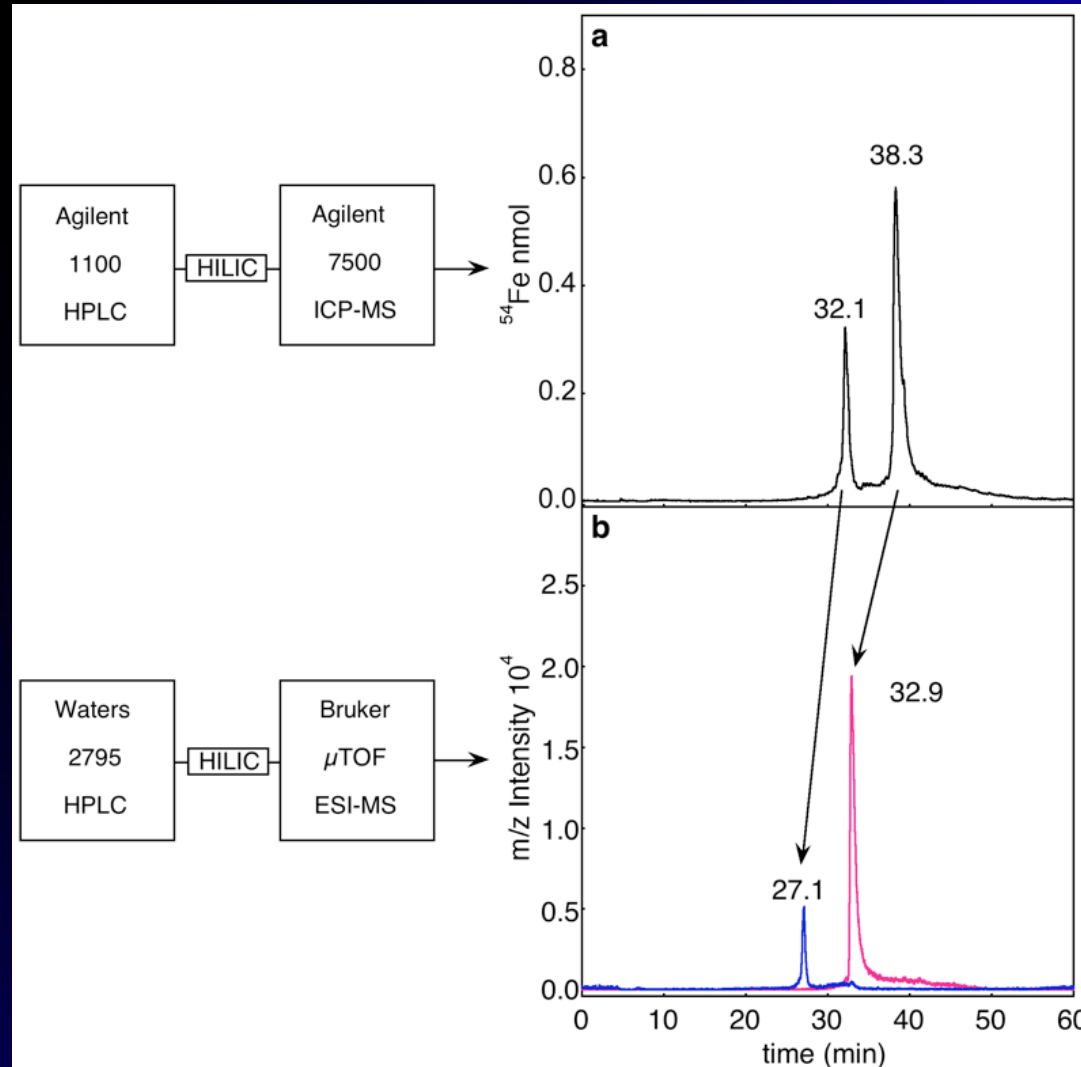
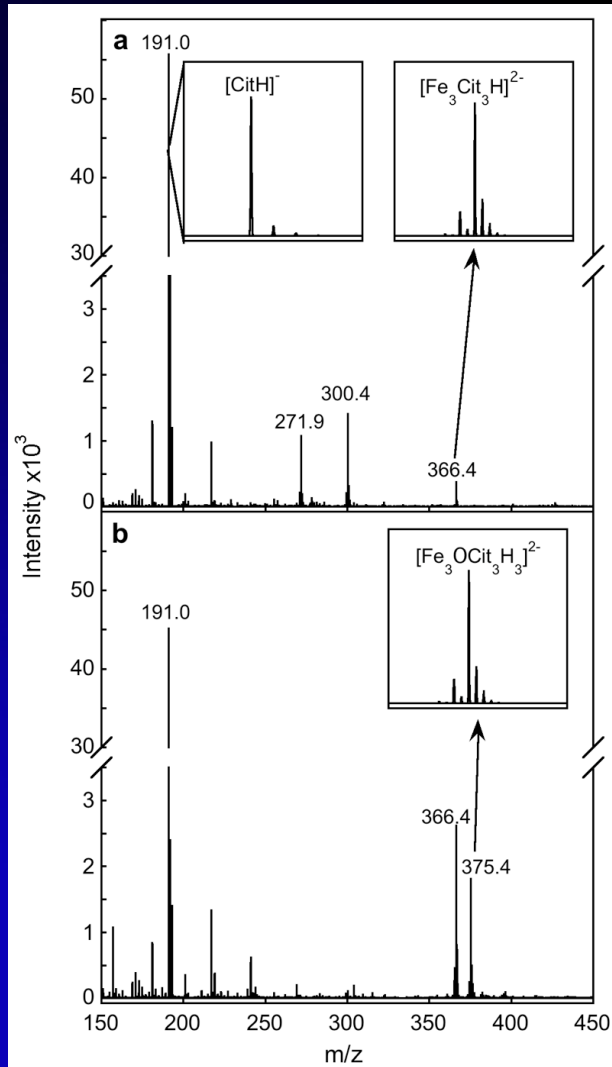
Implemented into software, the isotopic abundance pattern can be used to filter molecular formula candidates even at the 1 ppm level. In the mass spectrum between 700-900 Da, the isotopic abundance pattern is shown. The principal idea of



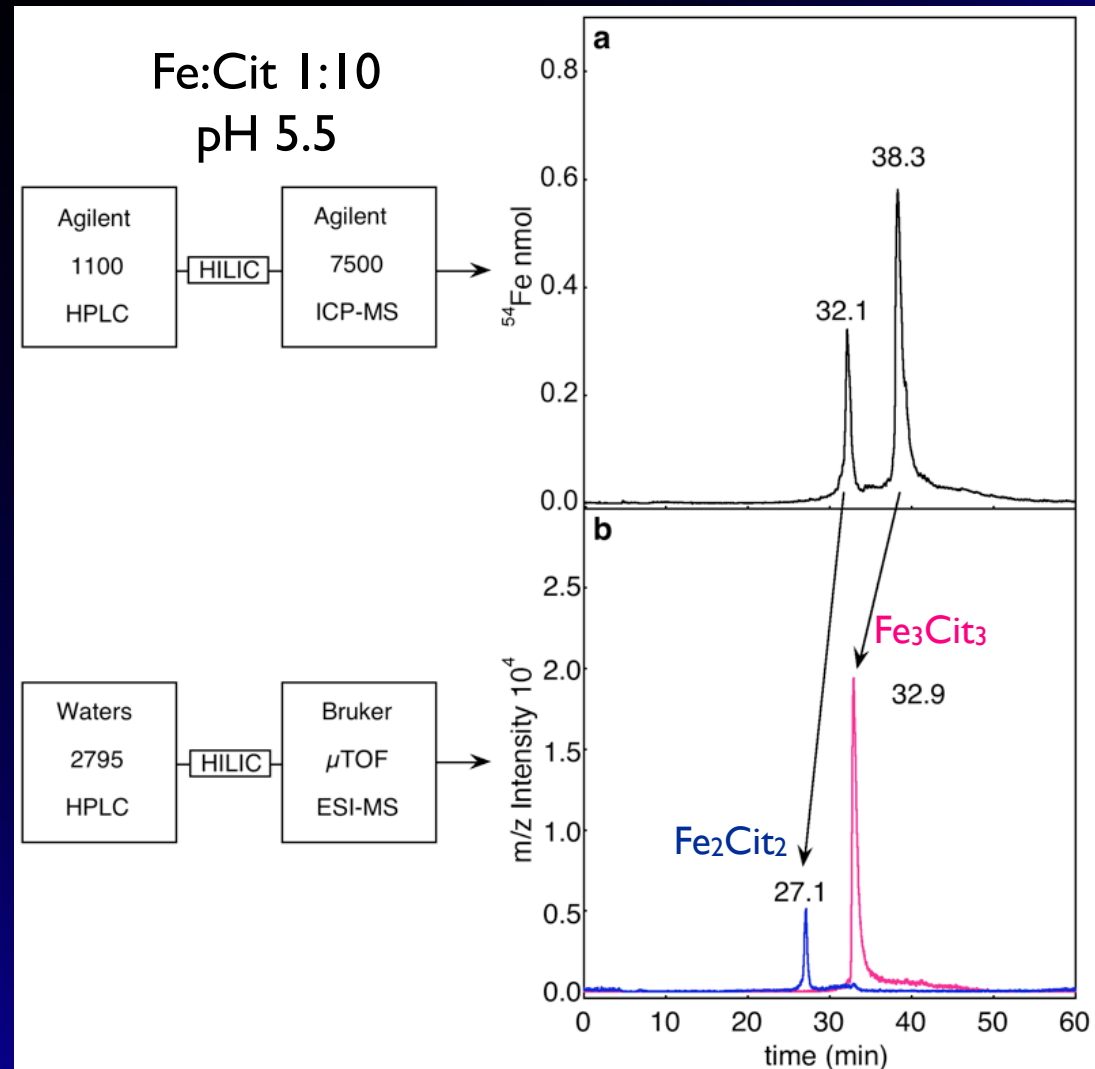
The isotopic abundances of the M+1 and M+2 ions can be used to filter molecular formula candidates. The isotopic abundances of the M+1 and M+2 ions in the example shows isotopic abundance pattern for siloxane. Other formulae can be excluded if the mass spectrometry

Kind and Fiehn, 2006





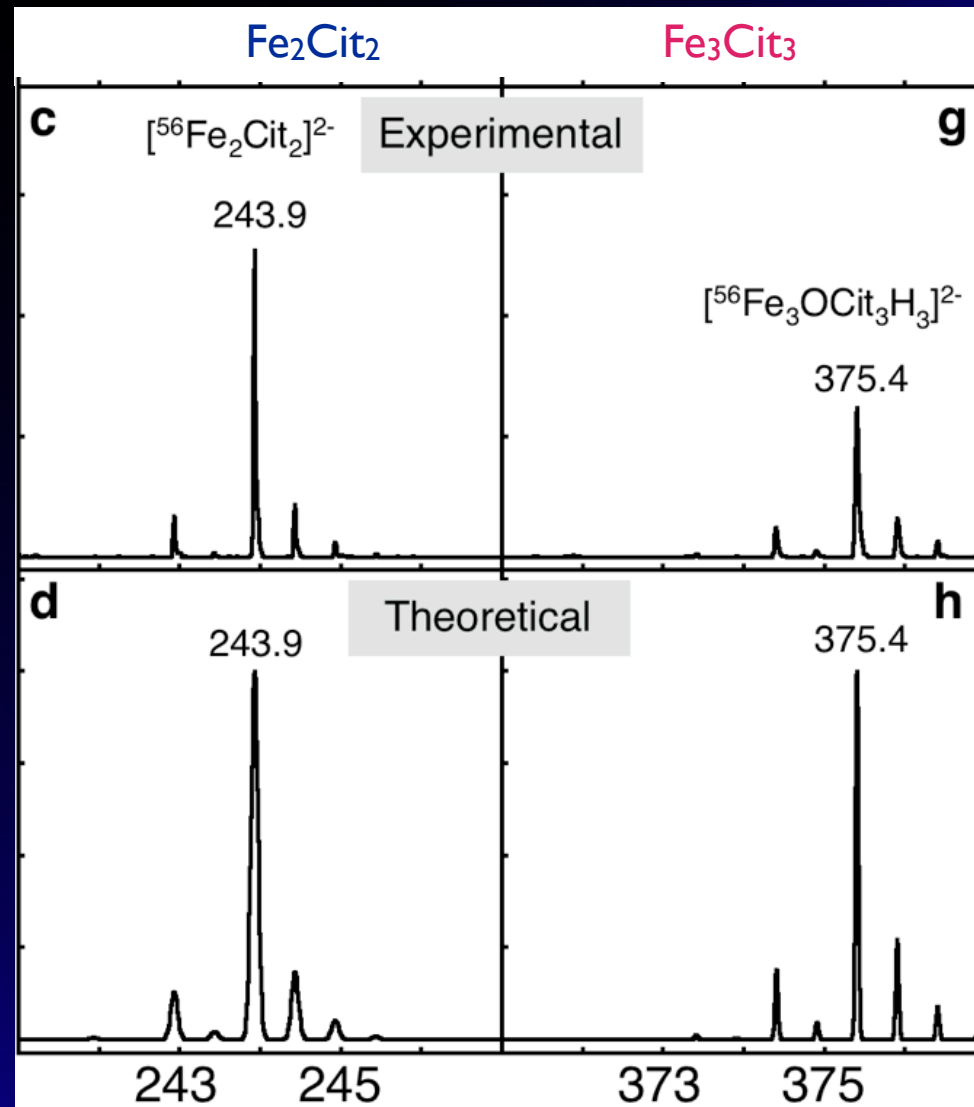
ESI and HPLC Optimization of Standard Solutions



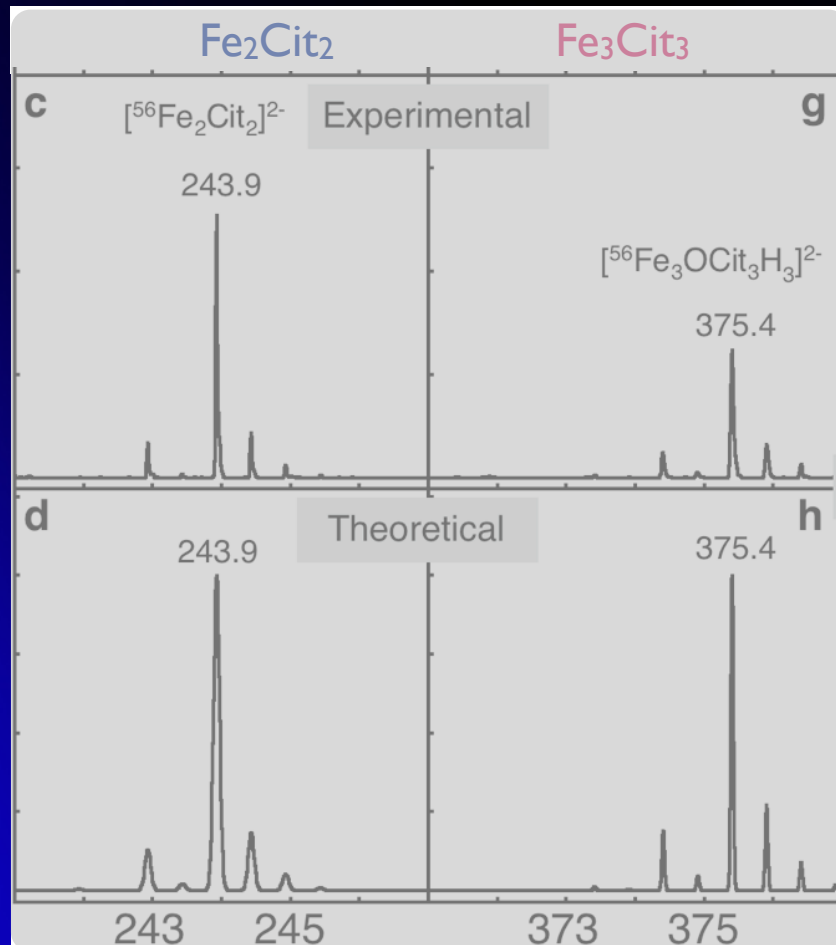
Isotopic signature

Fe-Cit in xylem
Rellan-Alvarez et al *in press*

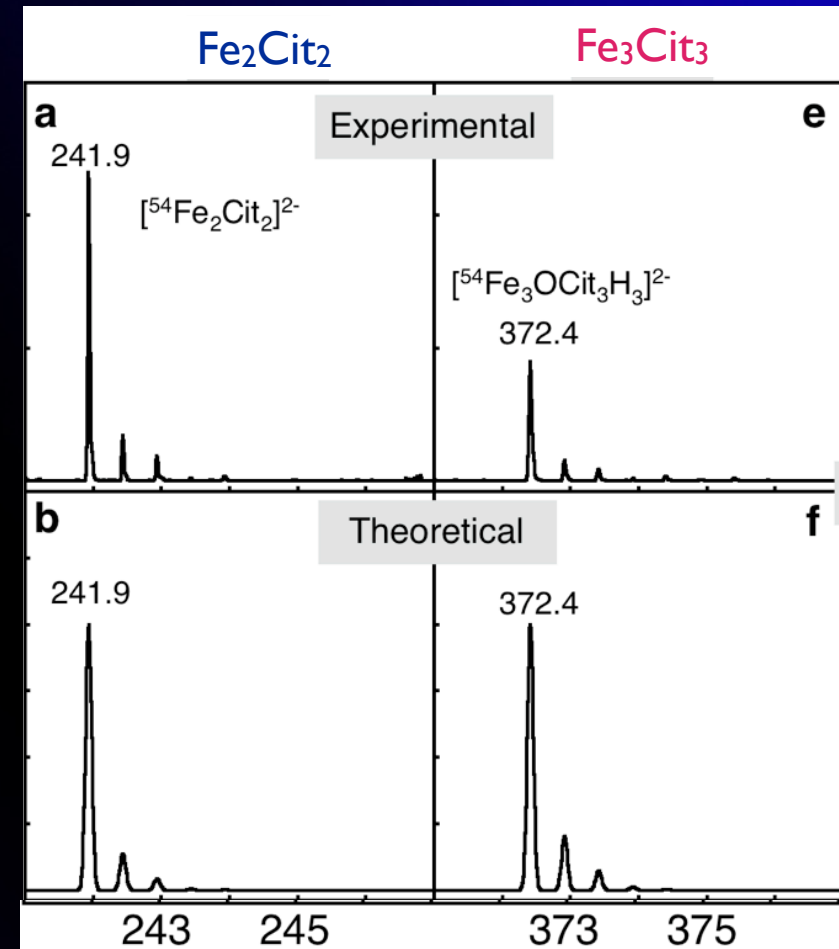
natFe



Isotopic signature



^{nat}Fe



^{54}Fe



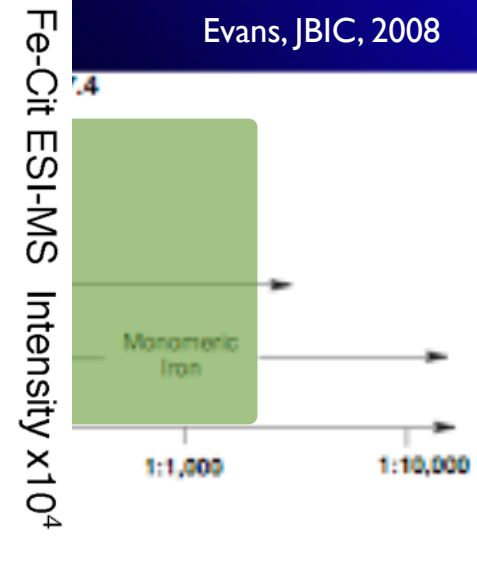
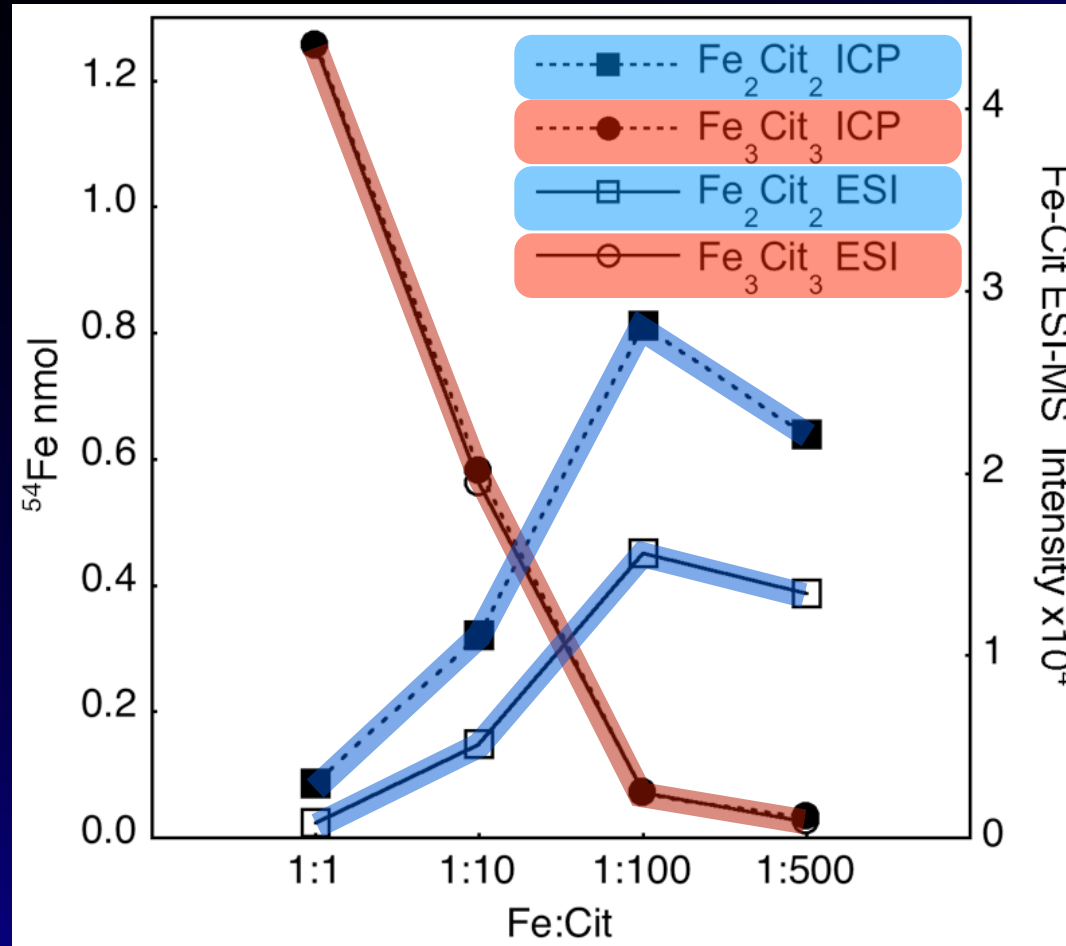
Isotopic signature and molecular formula determination

Measured m/z	⁵⁶ Fe/ ⁵⁴ Fe (Fe atoms) ^a	Charge ^b	Molecular formula	Calculated m/z	Error m/z (ppm)	SigmaFit™ value	Molecular ion
Standard solutions							
241.9362	-	-2	⁵⁴ Fe ₂ C ₁₂ H ₈ O ₁₄	241.9359	2.1	0.0234	[⁵⁴ Fe ₂ Cit ₂] ²⁻
372.4119	-	-2	⁵⁴ Fe ₃ C ₁₈ H ₁₃ O ₂₁	372.4127	2.3	0.0273	[⁵⁴ Fe ₃ Cit ₃ H] ²⁻
243.9311	7.4 (2)	-2	⁵⁶ Fe ₂ C ₁₂ H ₈ O ₁₄	243.9311	1.7	0.0165	[⁵⁶ Fe ₂ Cit ₂] ²⁻
375.4047	5.2 (3)	-2	⁵⁶ Fe ₃ C ₁₈ H ₁₅ O ₂₂	375.4057	1.7	0.0284	[⁵⁶ Fe ₃ OCit ₃ H ₃] ²⁻



Fe:Cit ratios drive the formation of Fe-Cit complexes

Fe-Cit in xylem
Rellán-Alvarez et al *in press*





Tomato Fe-deficient Plants
Resupplied with Fe 45 μM



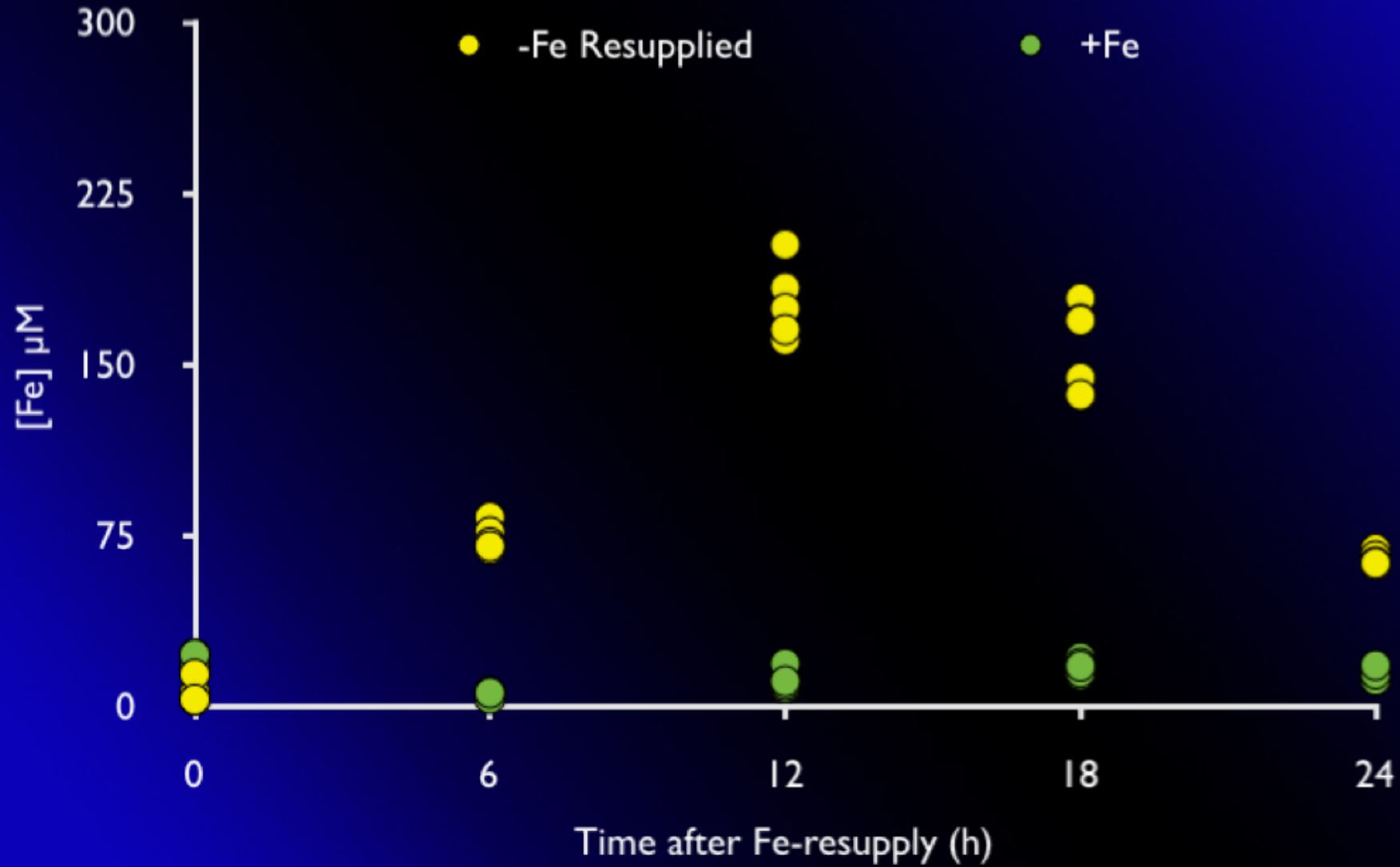
Xylem Collection

Fe-Cit in xylem
Rellan-Alvarez et al *in press*

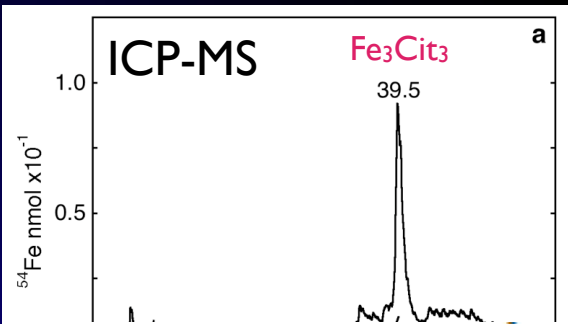


Xylem Fe Concentration

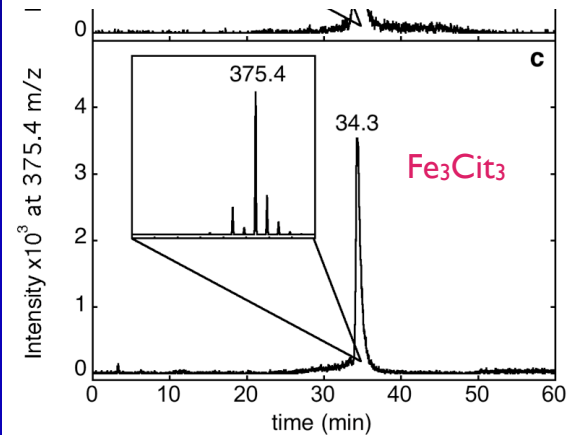
Fe-Cit in xylem
Rellan-Alvarez et al *in press*



Tomato Xylem Sap samples

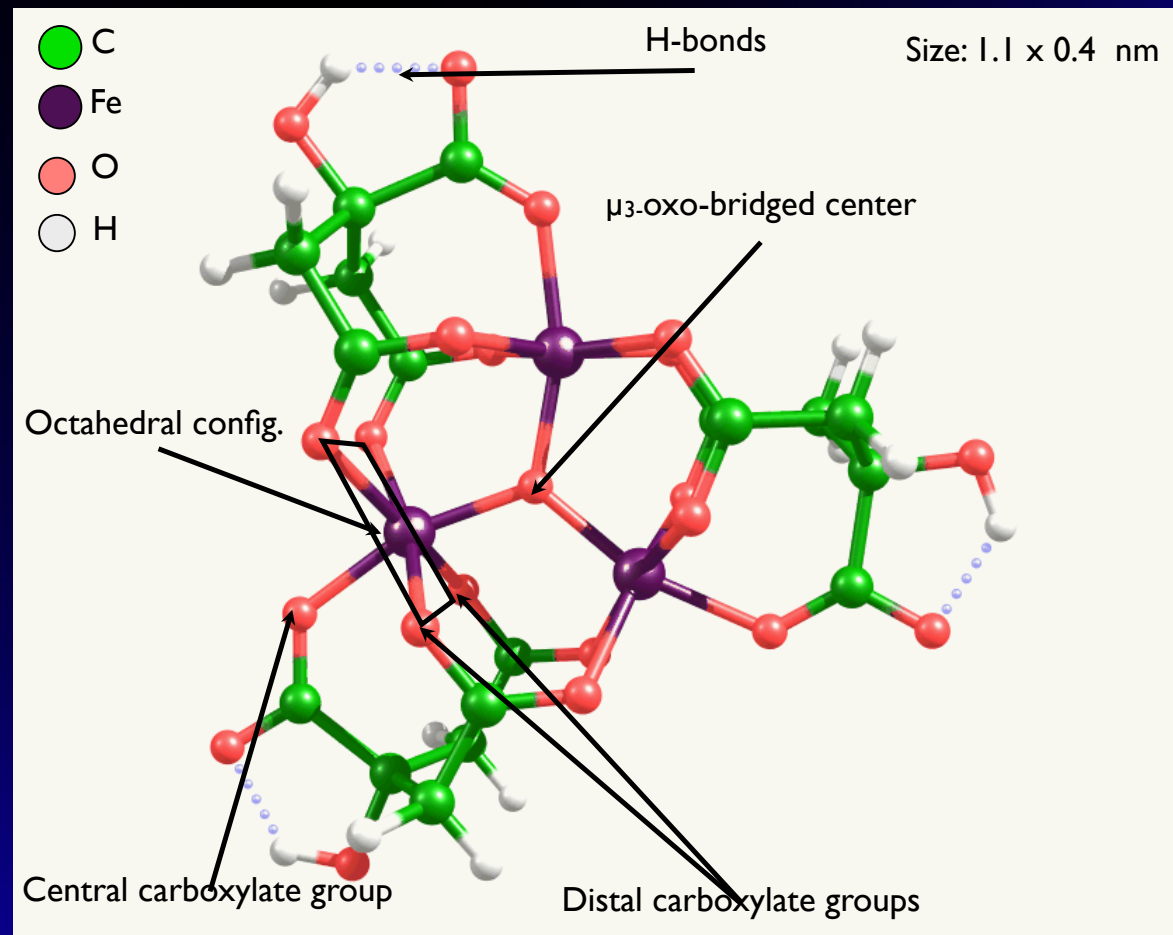


Measured m/z	$^{56}\text{Fe}/^{54}\text{Fe}$ (Fe atoms)	Charge ^b	Molecular formula	Calculated m/z	Error m/z (ppm)	SigmaFit TM value	Molecular ion
Xylem sap							
372.4097	-	-2	$^{54}\text{Fe}_3\text{C}_{18}\text{H}_{15}\text{O}_{22}$	372.4057	8.2	0.0283	$[\text{}^{54}\text{Fe}_3\text{OCit}_3\text{H}_3]^{2-}$
375.4045	5.56 (3)	-2	$^{56}\text{Fe}_3\text{C}_{18}\text{H}_{15}\text{O}_{22}$	375.4057	6.8	0.0197	$[\text{}^{56}\text{Fe}_3\text{OCit}_3\text{H}_3]^{2-}$



An oxo-bridged Fe₃Cit₃ complex

Fe-Cit in xylem
Rellan-Alvarez et al *in press*



Gràcies!



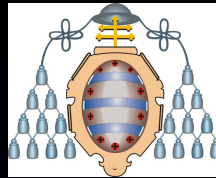
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