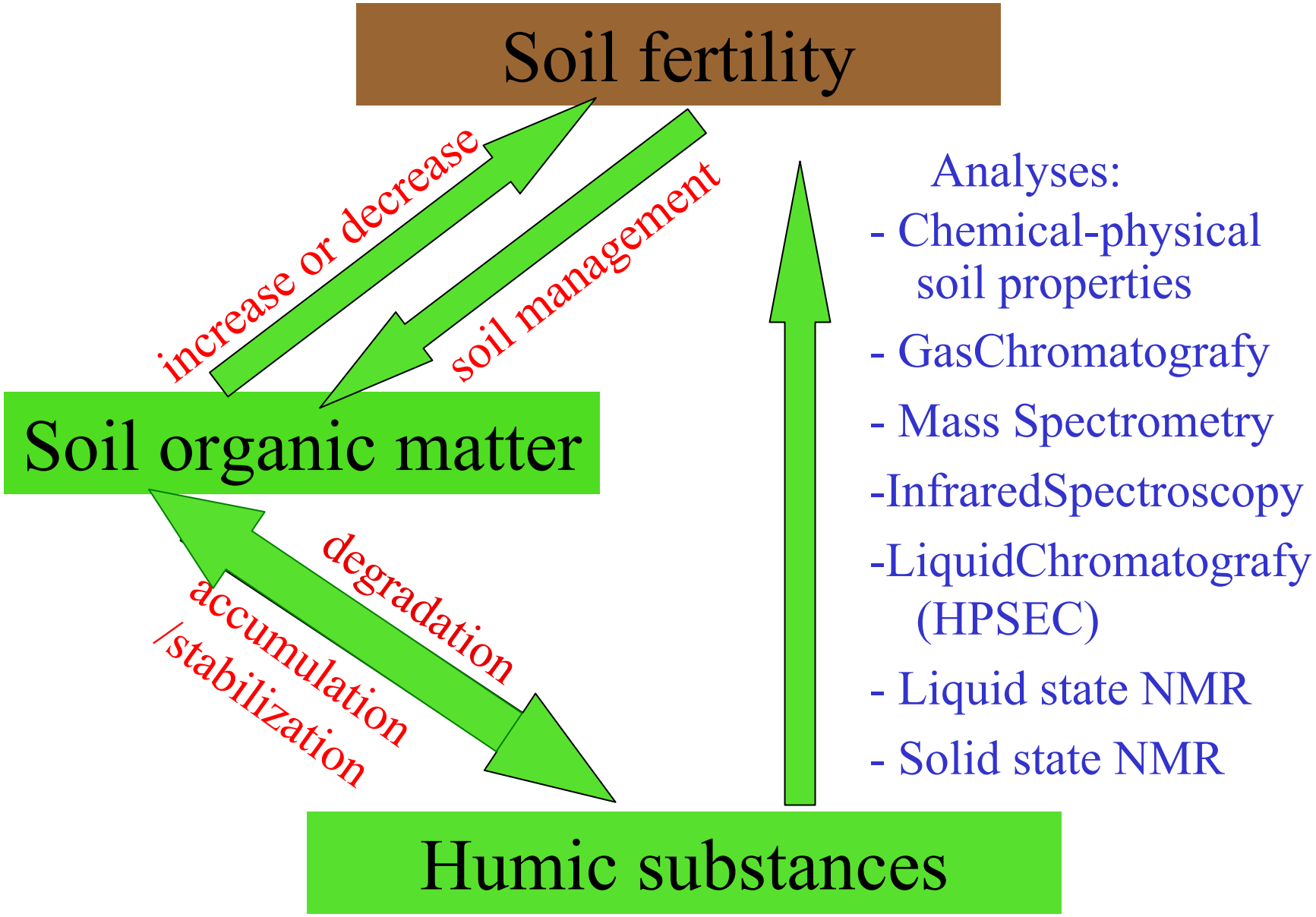
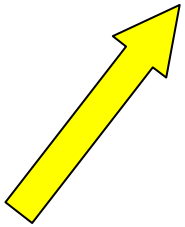

Soil fertility
increase or decrease
soil management
Soil organic matter
degradation
accumulation
/stabilization
Humic substances
Analyses:
- Chemical-physical soil properties
- GasChromatografy
- Mass Spectrometry
-InfraredSpectroscopy
-LiquidChromatografy (HPSEC)
- Liquid state NMR
- Solid state NMR

OBJECTIVE

*Molecular characterization of soil **humic acids** extracted after soil treatments with recycled organic biomass*

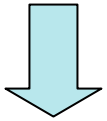
Spectroscopic analyses:

- IR-DRIFT (Diffuse Reflectance Infrared Fourier Transform)



molecular characterization

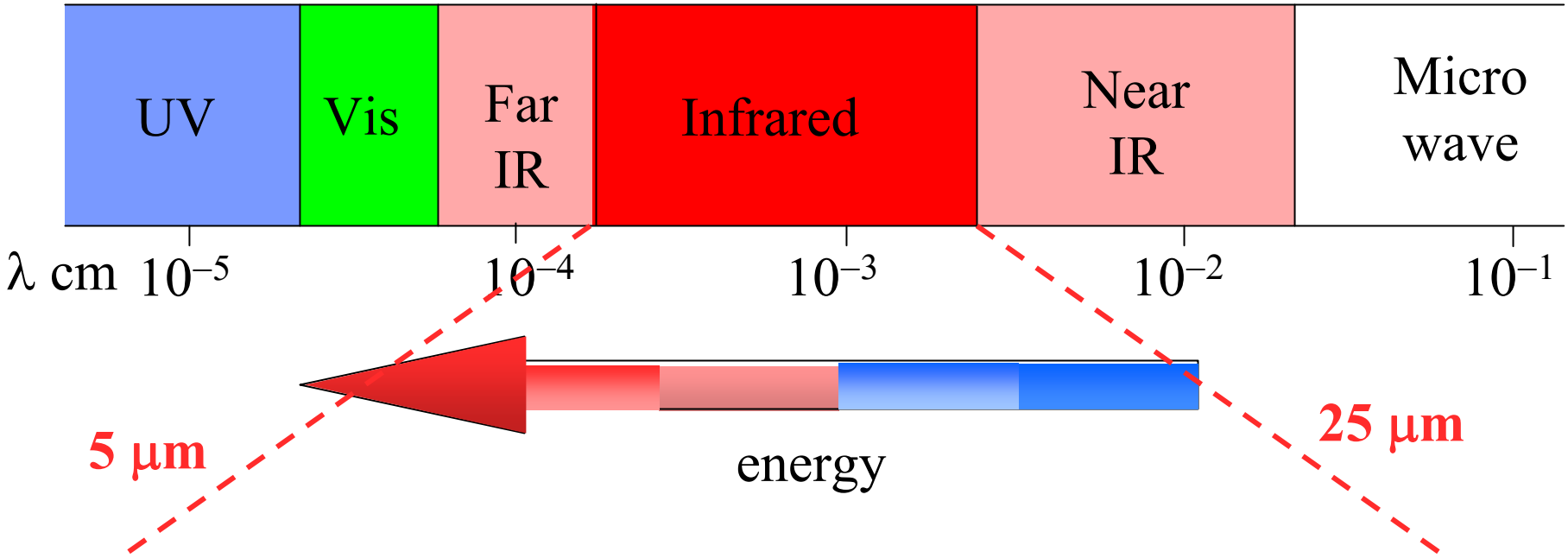
off-line pyrolysis with
TetraMethylAmmoniumHydroxide
(TMAH termochemolysis)



GasCromatografy MassSpectrometry

Infrared (IR) Spectroscopy

most spectroscopies techniques are based on the interaction between electromagnetic wave and the unkown molecule



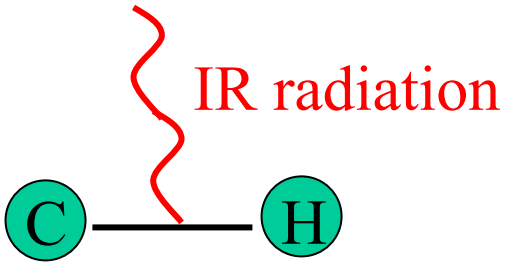
the energy associated with these wavelenght correspond to the *molecular vibration of chemical bonds energy*

Infrared Spectroscopy

wavelength λ (cm)

frequency $\nu = c/\lambda$ (Hz or s^{-1})

interaction with bond vibrational energy



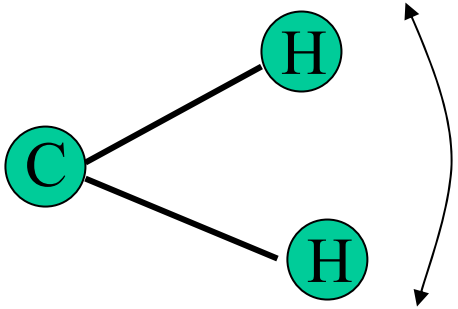
IR signals

stretching

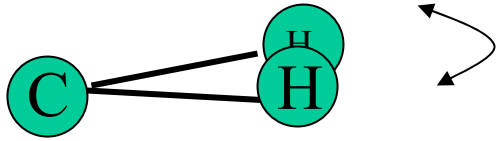
simmetrycal

asymmetrical

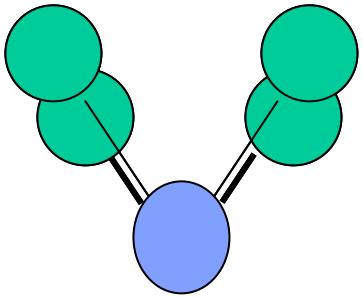
bending



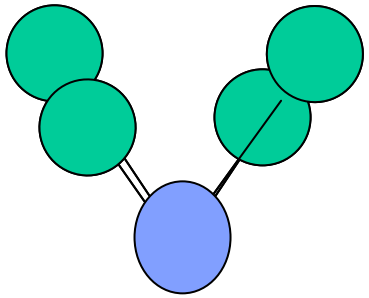
in-plane



out-of-plane

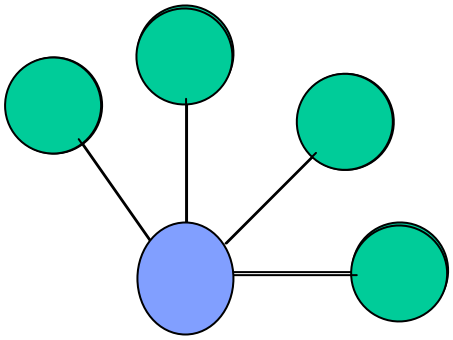


Simmetrycal stretching

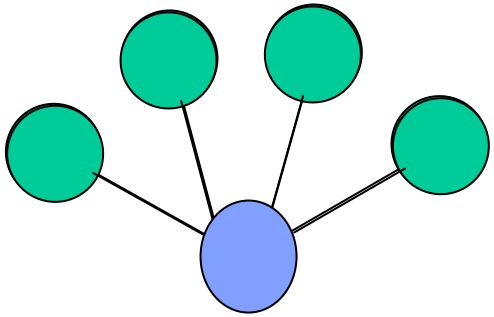


Asymmetrical stretching

In-plane bending

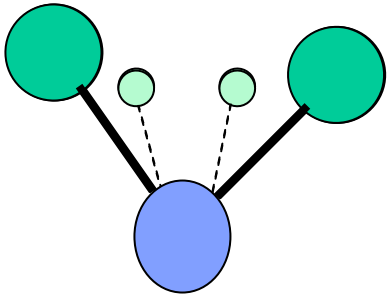


rocking

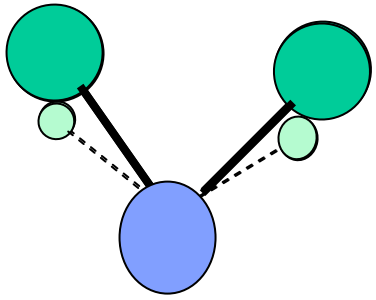


scissoring

Out-of-plane bending

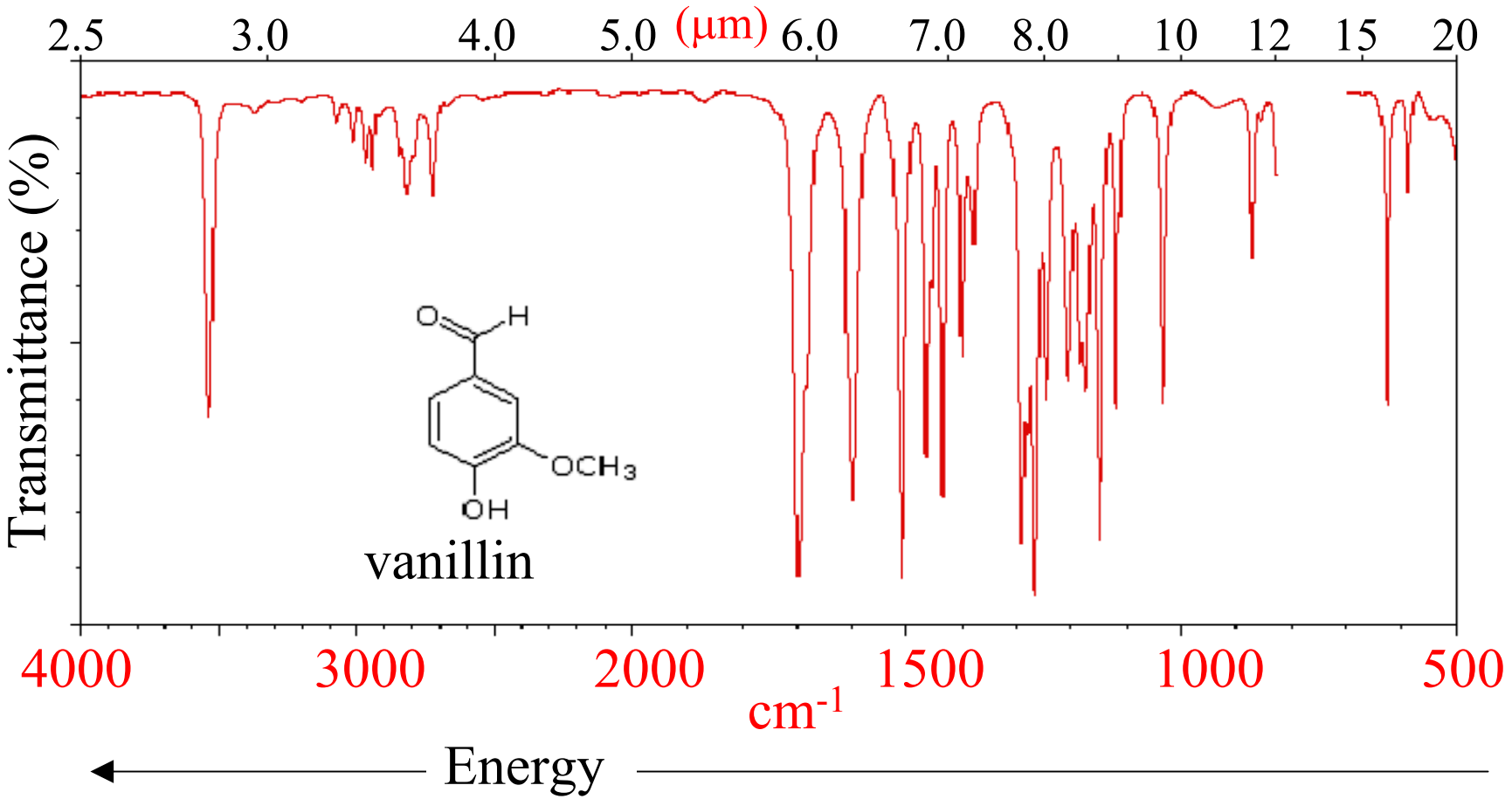


wagging



twisting

the IR spectra is a plot of transmittance intensity against wavelenghts



in modern IR instrument the wavelenght (λ) scale is replaced by wavenumber units defined as the inverse of (λ) in cm^{-1}

the wavenumbers are directly proportional to vibration energy and allow a linear plotting in the cm^{-1} units scale

the interaction between the incident IR ray and the chemical bonds produce typical absorption bands

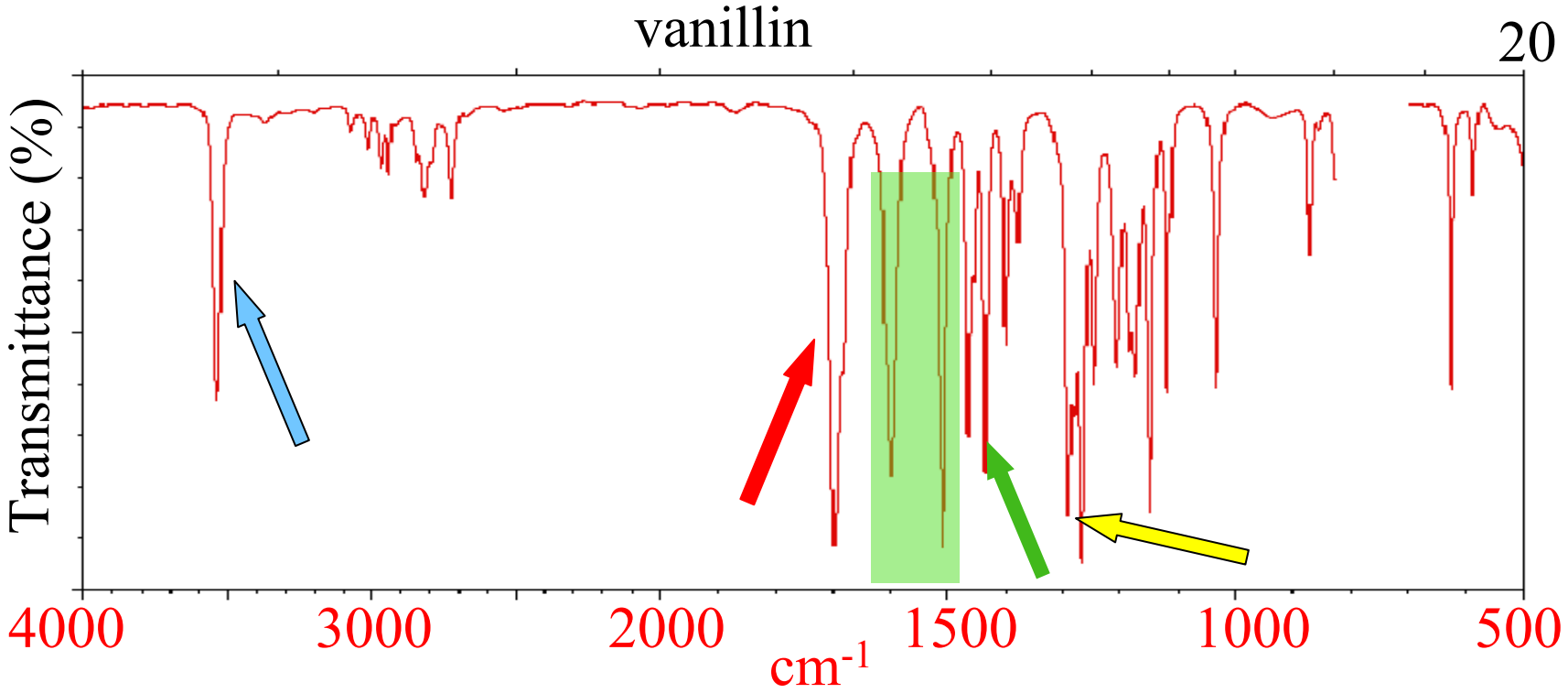
the main advantage of Infrared Spectroscopy is that each fuctional group has a unique frequency of absorption

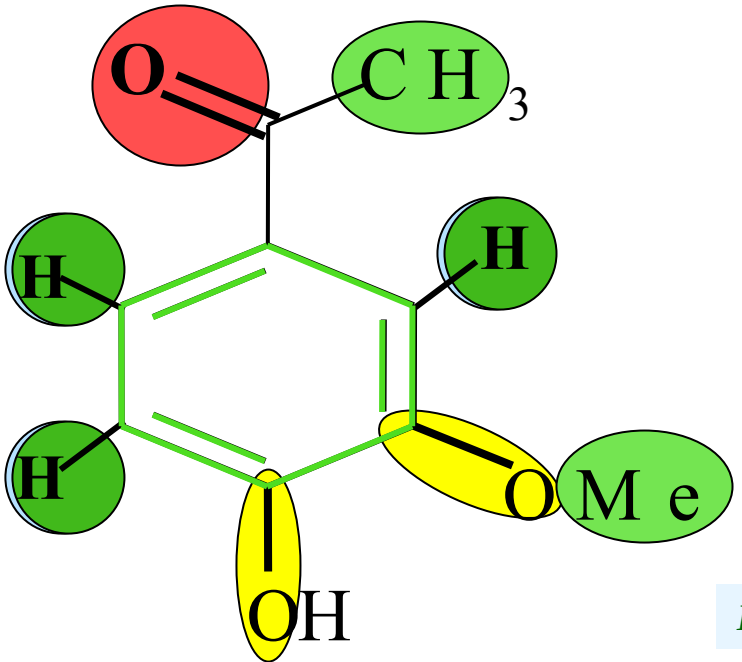
each functional group has the same absorption frequency irrespective to the overall molecule and to other groups

however in complex matrices such as SoilOrganicMaterial (SOM), humic substances, plant tissues etc. there is an overlapping of various functional groups

the interaction between vicinal functional groups (e.g. hydrogen bonding) and the presence of inorganic impurities (salt ions) modify the range of various absorption frequencies

Bond	/cm-1	attribution
R-C —H	3000-2850	stretching saturated alkanes
R-C — H	1480-1350	bending saturated alkanes
=C —H	3100-3000	stretching unsaturated alkanes or aromatic
=C —H	1600-1500	bending unsaturated alkanes or aromatic
R-O —H	3400-3000	stretching alcohols and phenols
R-O —H	1420-1330	bending alcohols and phenols
R-C —OR	1050-1300	stretching alcohols, phenols, ethers
R-C =O	1750-1710	stretching esters
R-C =O	1720-1680	stretching saturated/unsat. carboxylic acids
R-C =O	1680-1650	stretching amide (amide I band)
R-N —H	1620-1550	bending amide (amide I band)

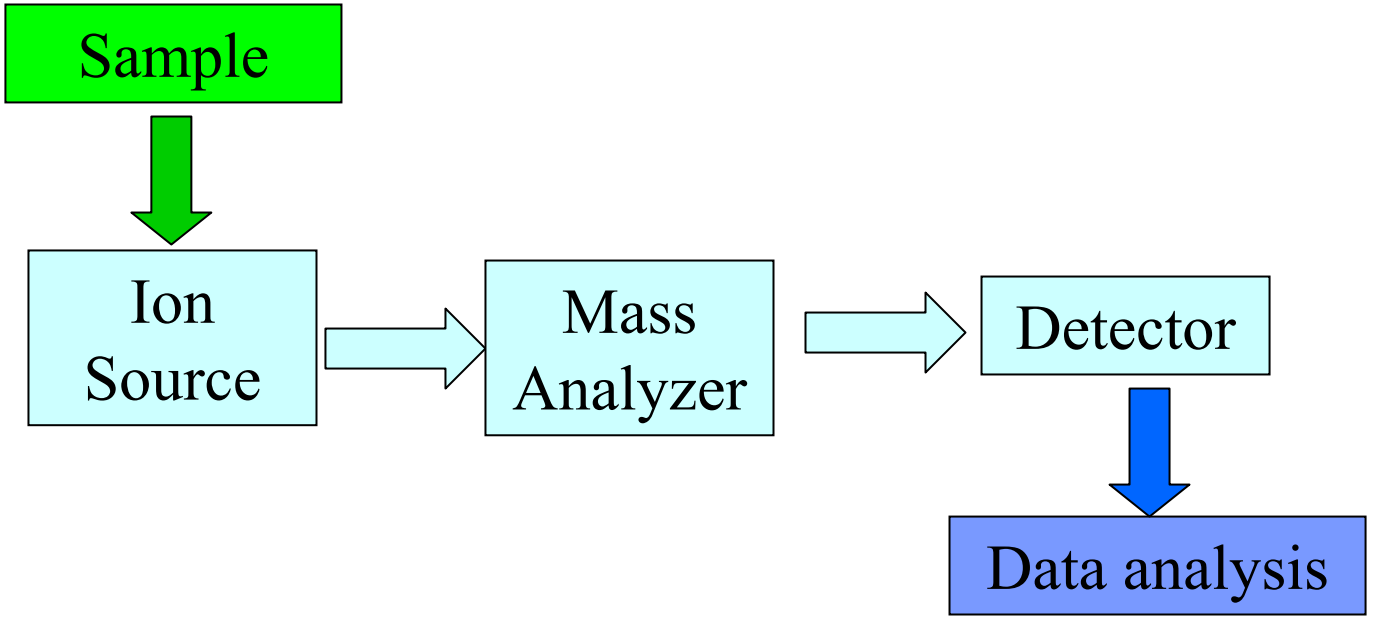

vanillin
Transmittance (%)
4000
3000
2000
1500
1000
500
cm-1


O
C H 3
H
H
H
O M e
OH

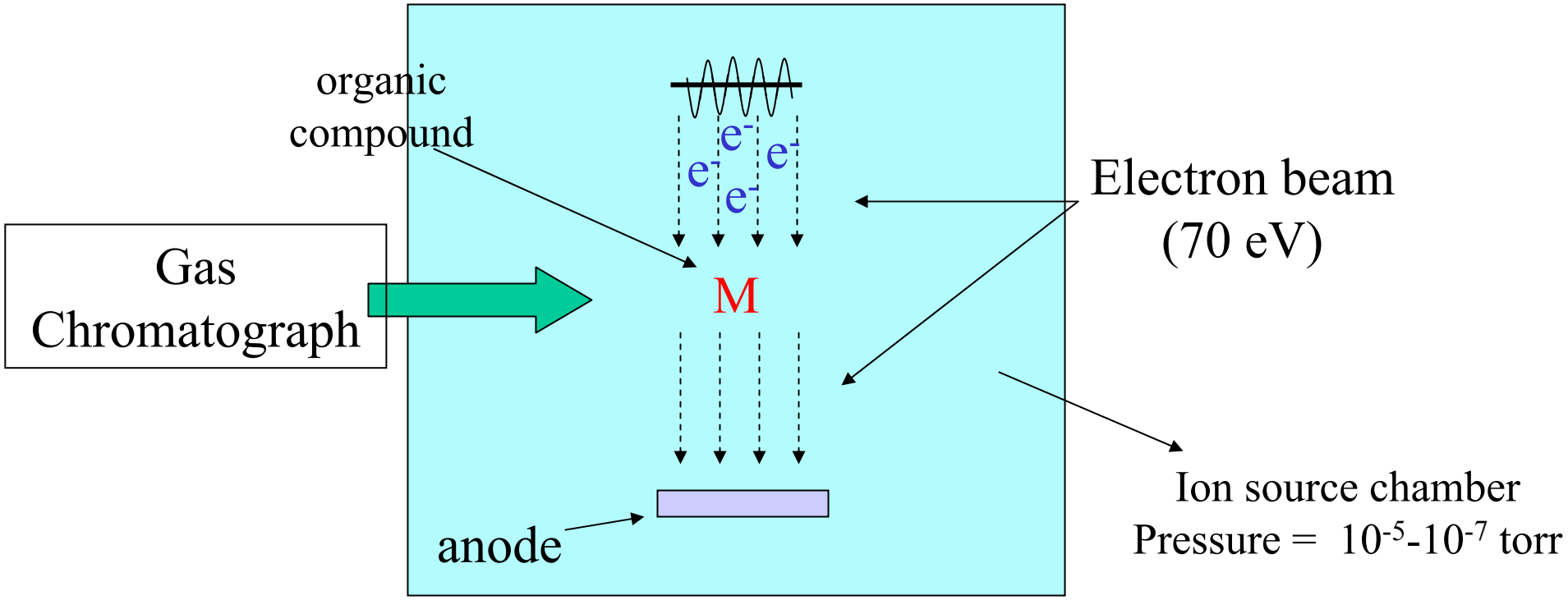
Electron Impact Mass Spectrometry (EI-MS)

the mass spectrometry analysis represent a powerful methods to identify the unknown organic compounds

the various techniques are based on the breaking of organic molecules in small charged fragments (ions); the detection and the analysis of various fragments (ions) allow the identification of the original organic compounds



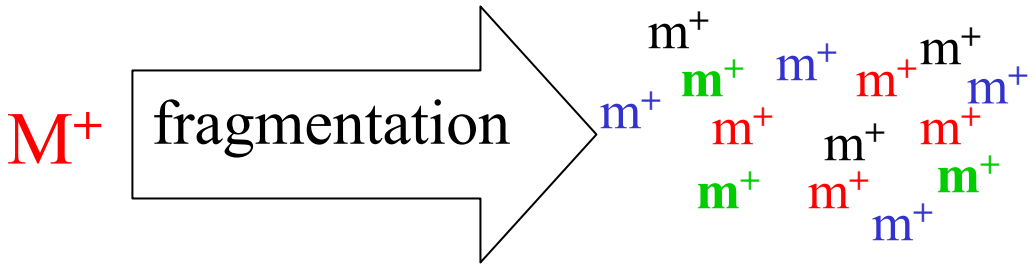
In the Electron Impact MS the organic compound is introduced in the ion source (under high vacuum) and bombarded with an high energy electron beam (70 eV)



$M + e^- \longrightarrow M^+ + 2\ e^-$

the collision of electron beam transfer to the organic compound a large energy content thereby releasing one electron and producing a positively charged radical

the positive charged radical is highly unstable and it start immediately to loss the excess of energy by breaking down (fragmentation) in small positively charged fragments with lower masses (positive mass fragments) of smaller molecular weight



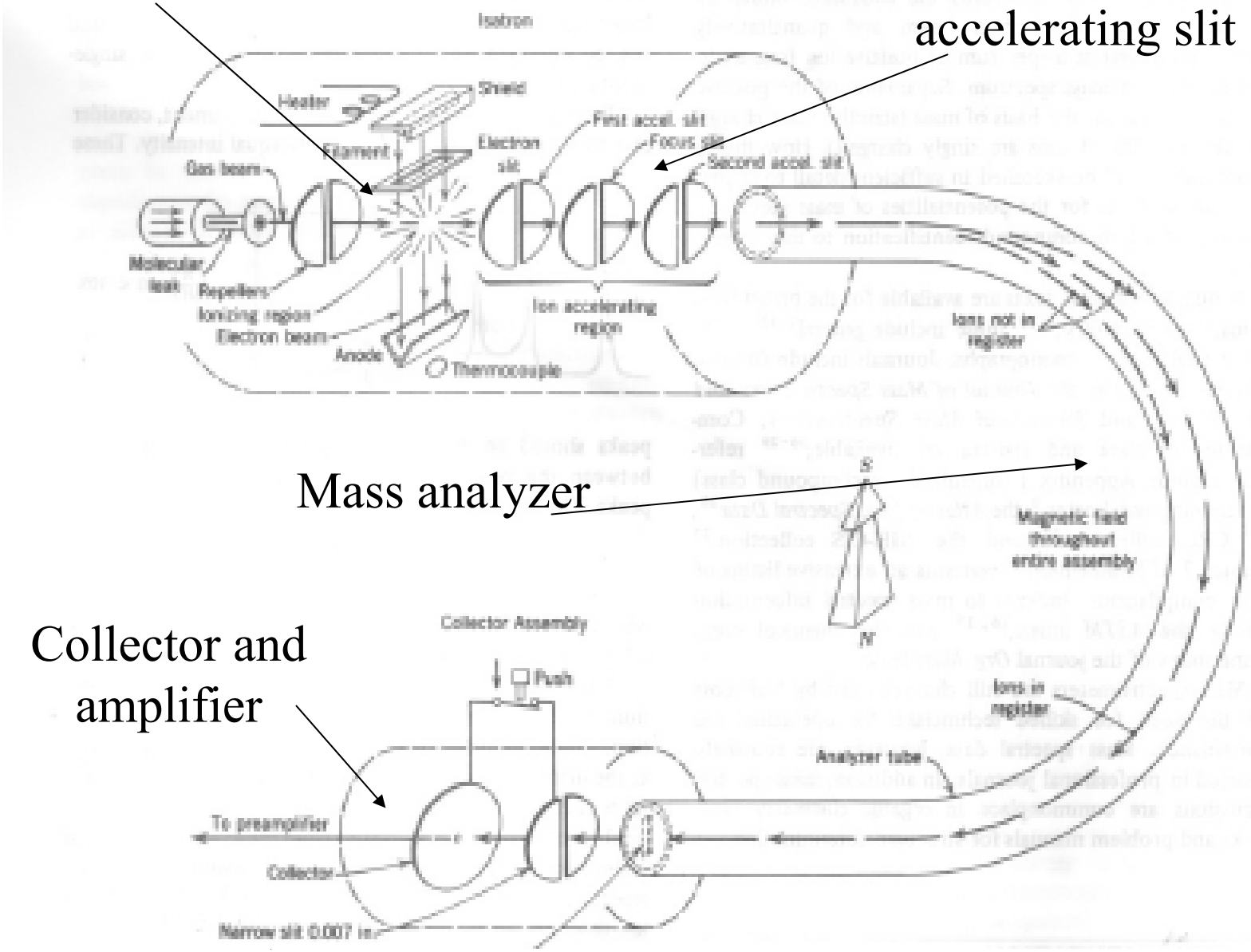
the various mass fragment (ions) are then collected by electronic lens and accelerating slit and pushed in the mass analyzer which provides a separation with respect to their molecular weight

the various fragments are then collected by sampling at regular interval (usually 1 second) and forwarded to the detector system

Mass spectra

Ion source

Electronic lens and accelerating slit



Mass analyzer

Collector and amplifier

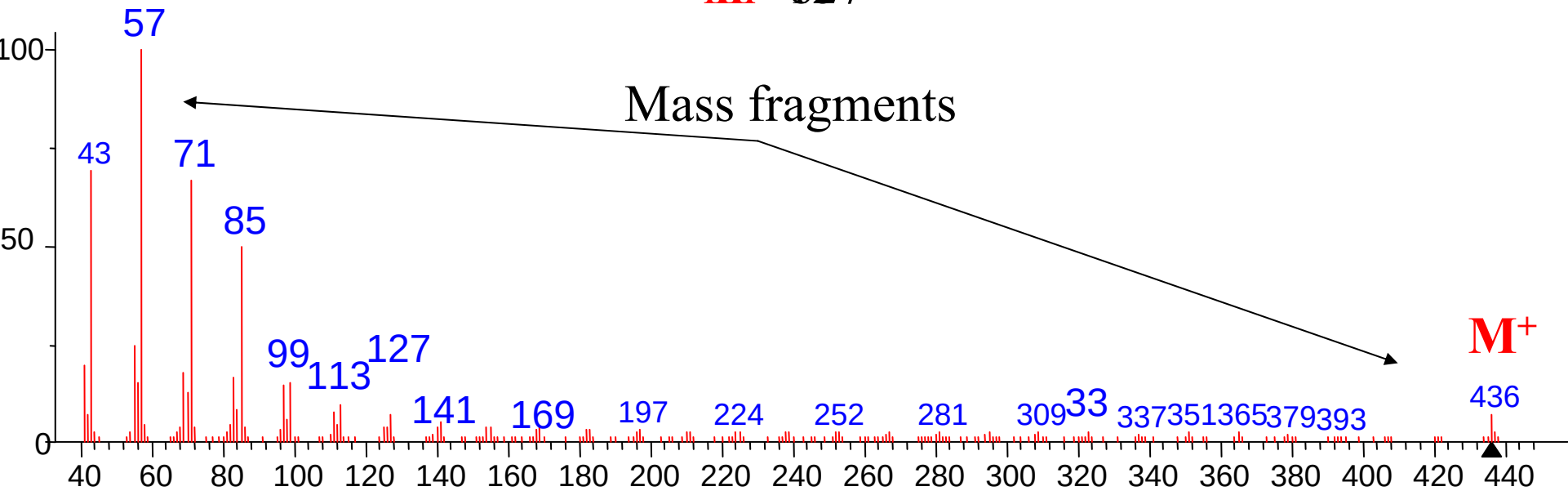
Example of mass spectra: linear hydrocarbon /alkane

Hentriacontane $C_{31}H_{64}$ m.w. 436

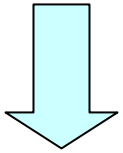
the higher masses are energetically unstable and and have a short lifetime

the smaller masses are enrgetically stable and hence are more abundant

Mass spectra



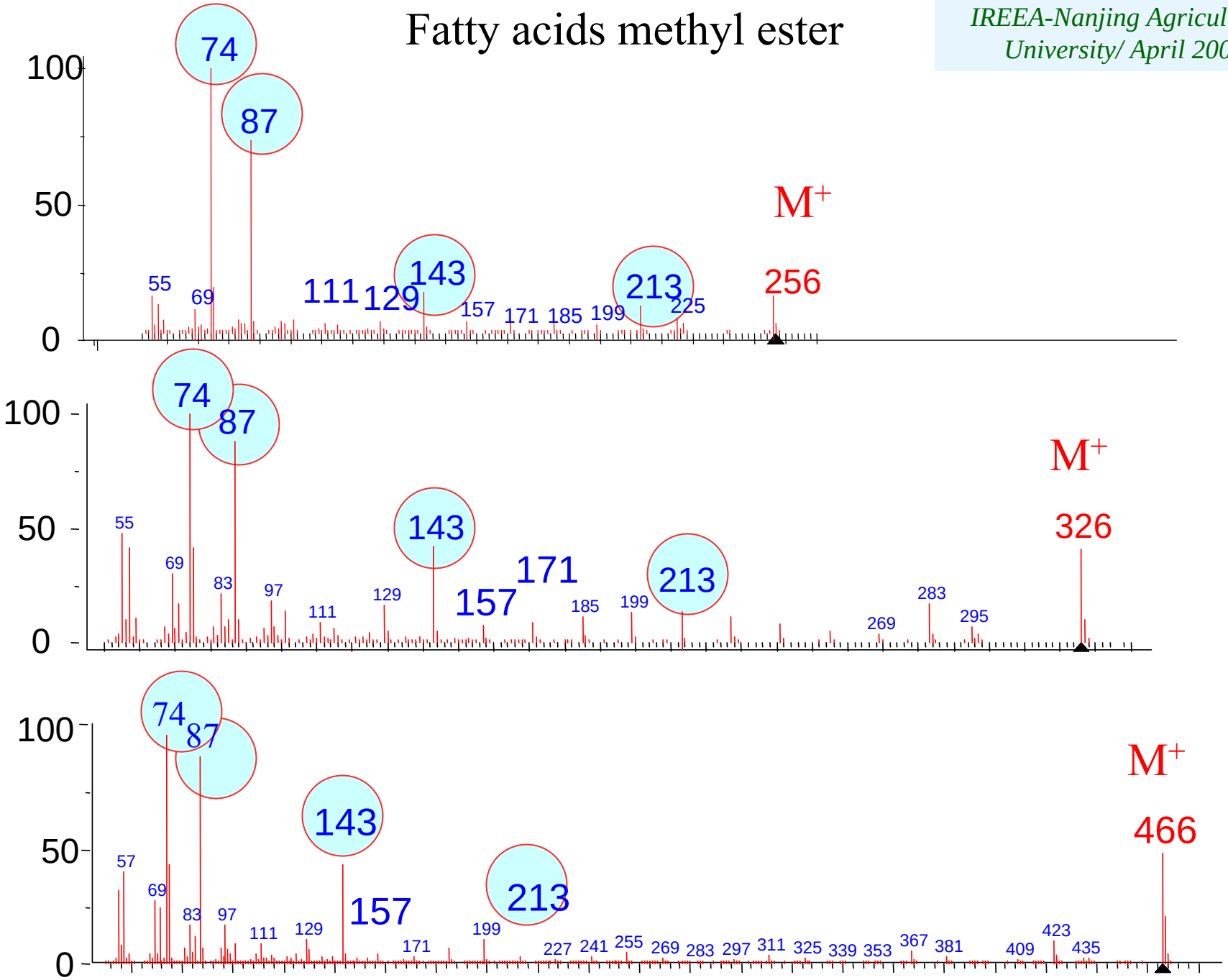
the main advantage of EI-MS is that each compound classes have the same fragmentation pattern



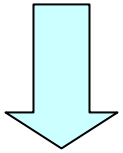
the mass spectra are fingerprint of specific organic compounds classes

Fatty acids methyl ester

IREEA-Nanjing Agricultural University/ April 2008



the main advantage of EI-MS is that each compound classes have the same fragmentation pattern



the mass spectra are fingerprint of specific organic compounds classes

the mass spectra obtained from EI-MS technique greatly simplify the identification of unknown organic molecules

the modern MS spectrometer are in fact associated with software for the interpretation of mass spectra

the software allow a quick comparison between the mass spectra of unknown compound with the mass spectra of standard compounds of library database

OBJECTIVE

*Molecular characterization of soil **humic acids** extracted after soil treatments with recycled organic biomass*

Spectroscopic analyses:

- IR-DRIFT (Diffuse Reflectance Infrared Fourier Transform)

molecular characterization

off-line pyrolysis with
TetraMethylAmmoniumHydroxide
(TMAH termochemolysis)

GasCromatografy MassSpectrometry

The recycled organic biomass (compost) was composed by the following organic residues

45% urban solid waste 40% plant residues (mais) 15% plant trimming

the recycled organic biomass was produced through a composting process made of a common oxidation period (*active phase)* of 30 days, followed a stabilization period (*curing phase)* of of 120 days

30 t ha^{-1} of recycled organic biomass were added to soil for 4 years

soil humic acids were then extracted from soil at the following intervals

4^{th} year (HA 0 year) 5^{th} year (HA 1 year) 6^{th} (HA 2 year)

Humic acid extraction

200 g soil

Soxhlet extraction with ethyl ether

1M NaOH + 0.1M $Na_4P_2O_7$ shaken overnight (1 liter)

centrifugation 7000 rpm

supernatant

pellets

filtration quartz filter 0.45 μ

washing with deionized water till pH7

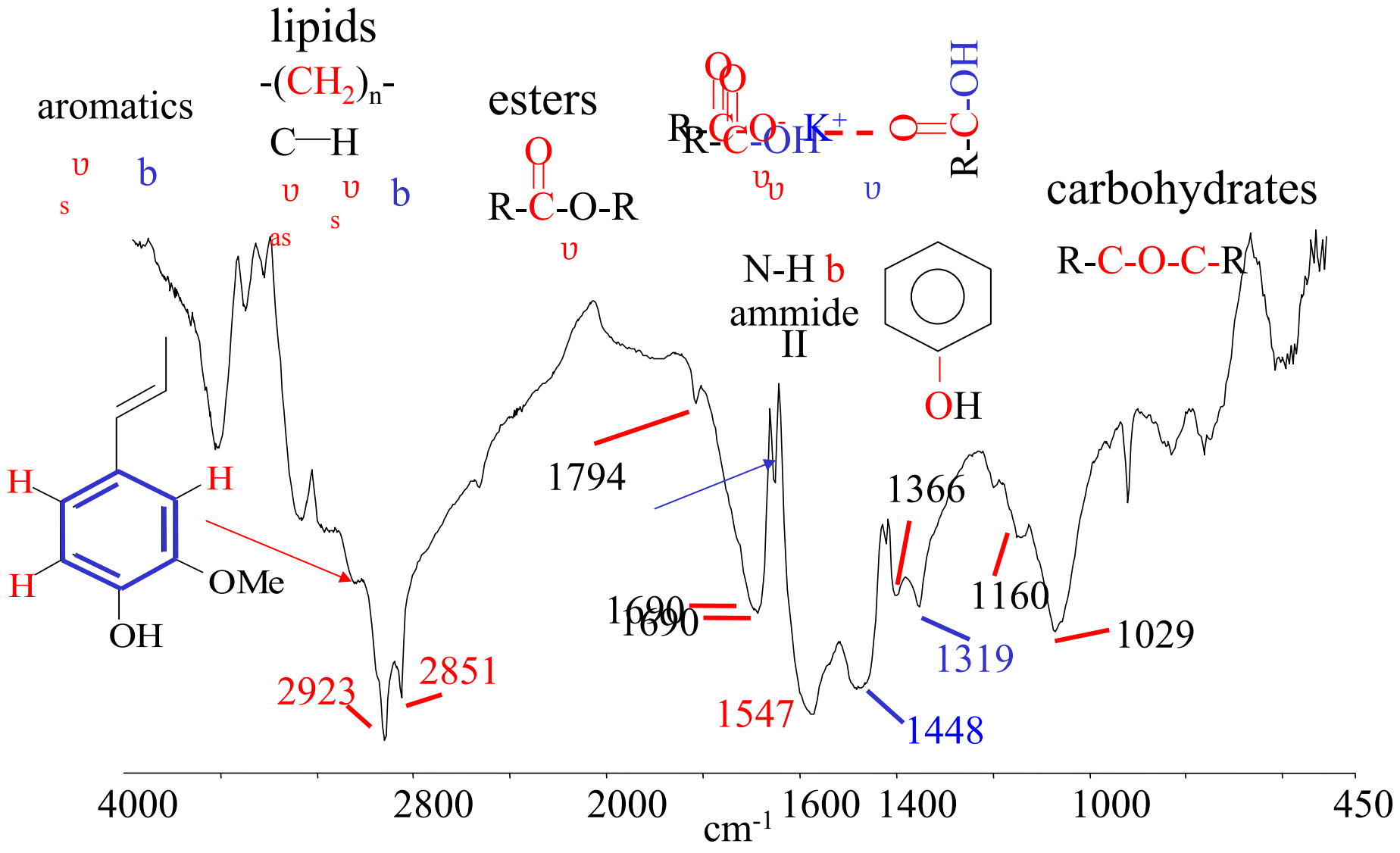
pH 1 with HCl

humic acids

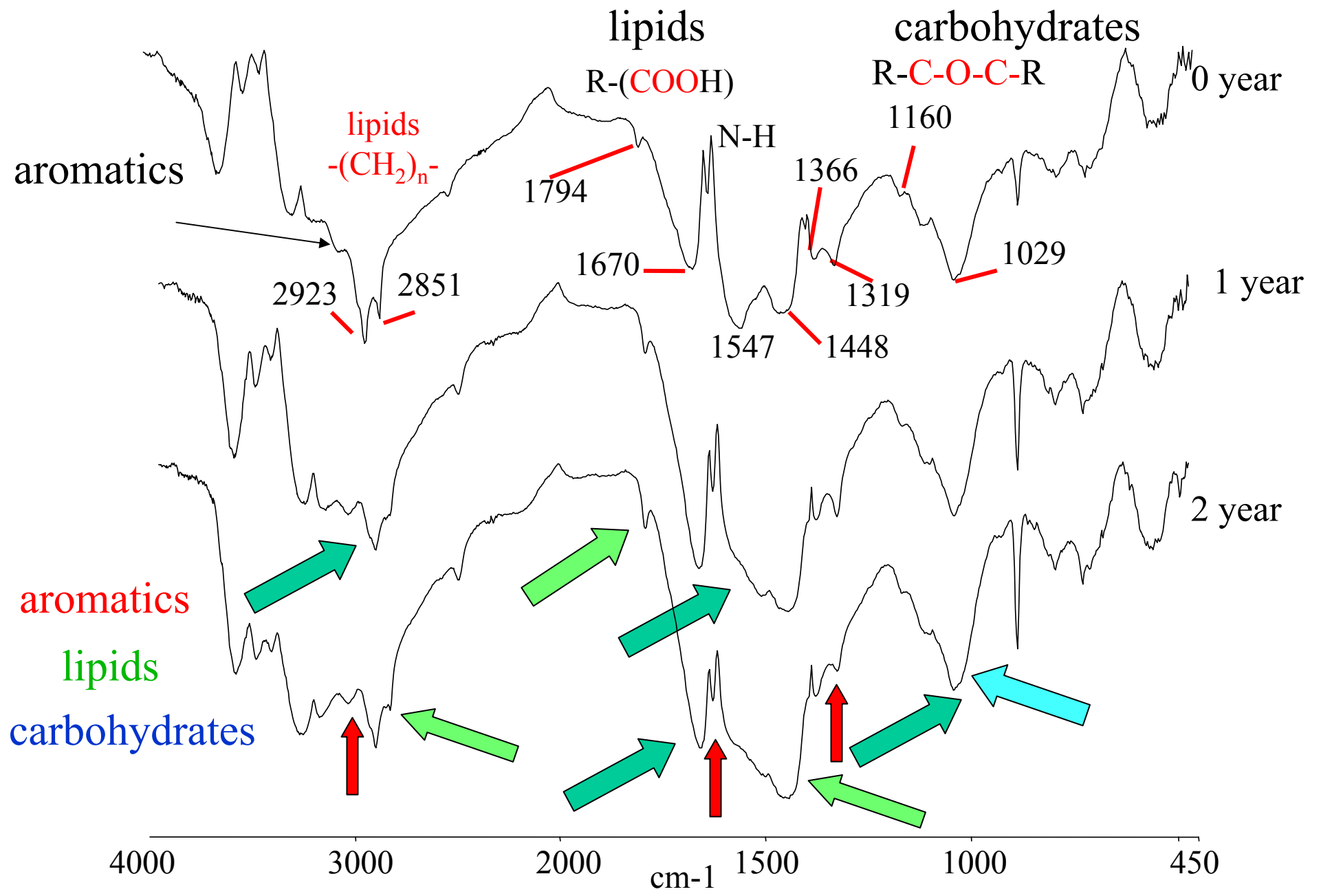
purification with HF/HCl solution

dialysis

IR-spectra HA 0 year



IR spectra of soil Humic acids



IR results

- the infrared spectra of humic acid extracted after soil addition with organic fertilizer revealed a composition dominated by lipid compounds and carbohydrates with aromatic and peptidic moieties

- after one year the spectroscopy data suggested a variation in the humic acid composition represented by a large loss of alkyl components and a significant decrease of biolable compounds such as peptidic material

- after two year the IR spectra of soil humic acid was characterized by a decrease of carbohydrates content. The final composition of humic acids revealed a prevalence of stable components represented by residual alkyl chains, aromatic compounds, ester and fatty acids and carbohydrates

Pirolysis-TMAH (termochemolysis)

- thermochemolysis technique has been increasingly used in the last 20 years for the analysis of complex organic matter matrices such as humic substances, SOM, plant tissues etc.

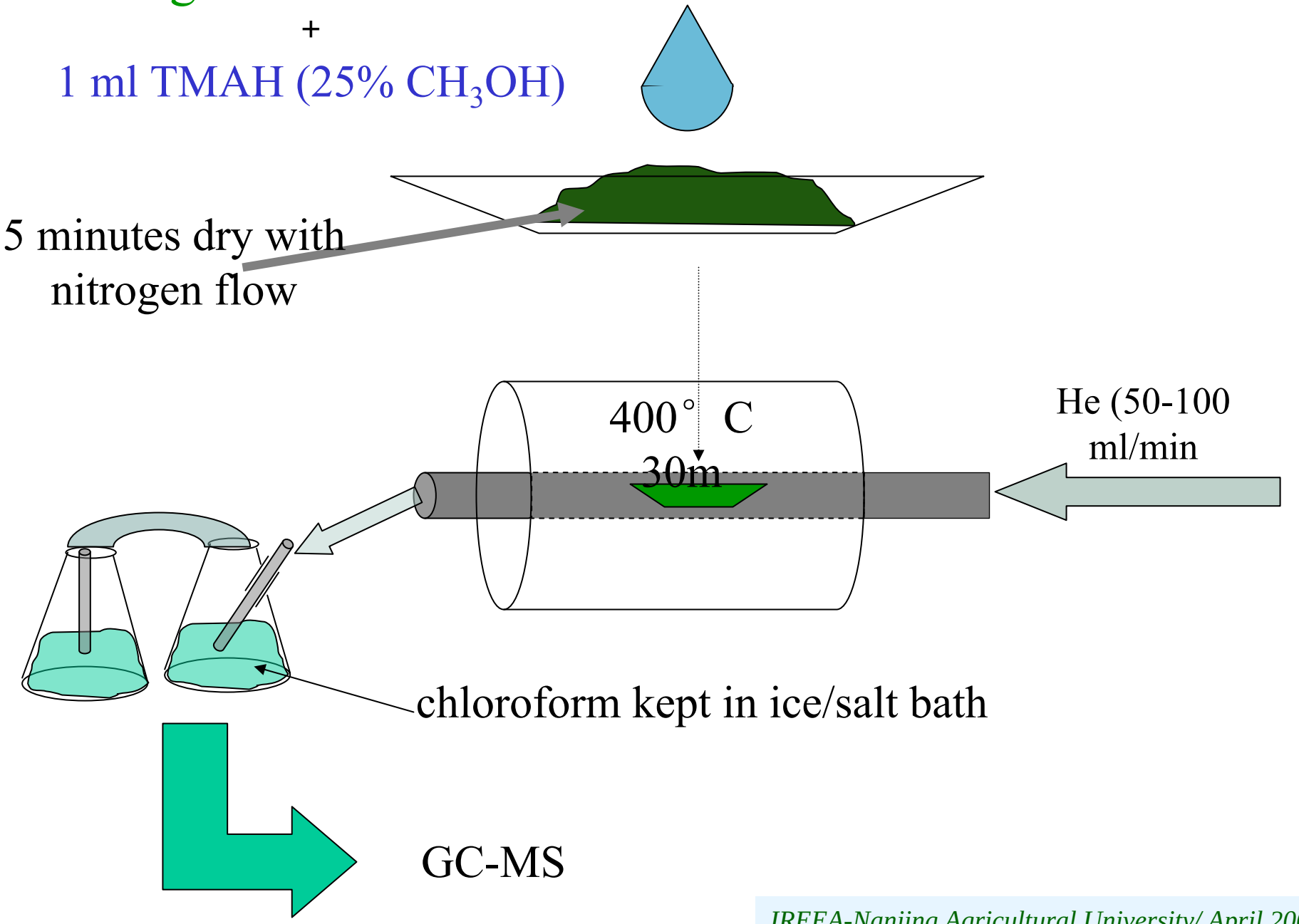
- this technique is based on the contemporaneous application of high temperature (400-700 ˚C), and alkylating reagents (TMAH), under inert gas atmosphere (He_2), for the breakdown of the covalent bonds that link together the organic matter components (C-C bonds, ester bonds, ether bonds)

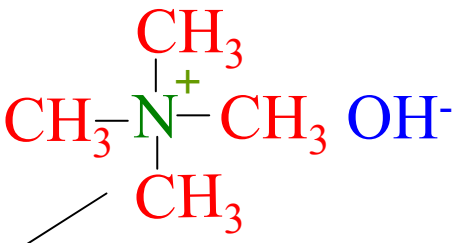
- the breakdown of covalent bonds simplify the organic materials releasing low molecular weight components that are hence amenable for the Gas Chromatographic Mass Spectrometry analysis

1 g Humic Acids

+

1 ml TMAH (25% CH_3OH)



Tetra Metil Ammonum Hydroxide (TMAH) 

(da Challinor 2001 JAAP Vol. 61)

Long chain ester → $R\text{-}COOR + (CH_3)_4N^+ OH^-$

$ROH + RCOO^-$

$ROH + (CH_3)_4N^+ OH^-$

$RO^- (CH_3)_4N^+ + H_2O$

$(CH_3)_3N + RO\,CH_3$

Methyl ether

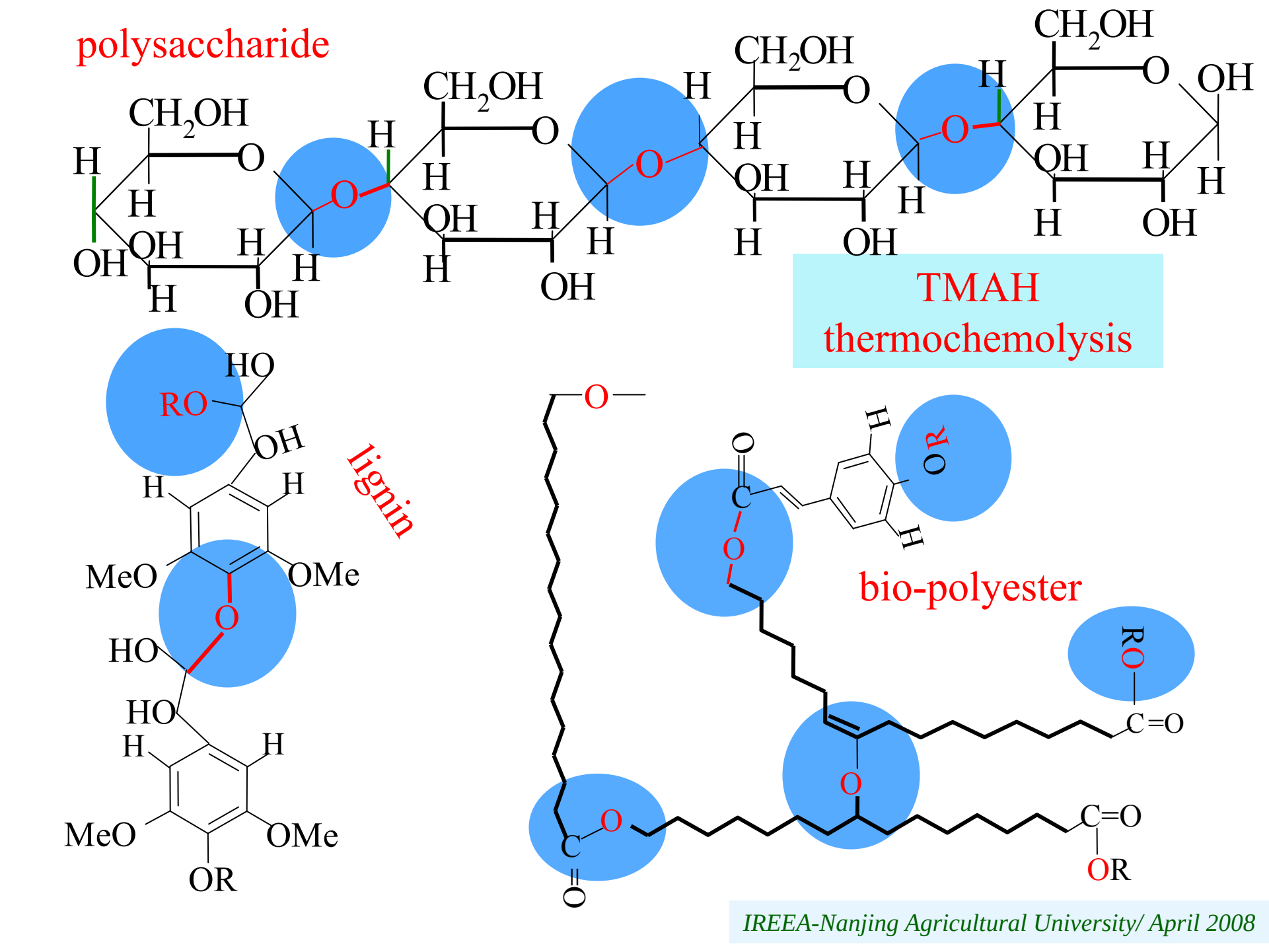
$RCOO^- + (CH_3)_4N^+$

$RCOO^- (CH_3)_4N^+$

$RCOOCH_3 + (CH_3)_3N$

Methyl ester

GC-MS


polysaccharide
TMAH
thermochemolysis
lignin
bio-polyester
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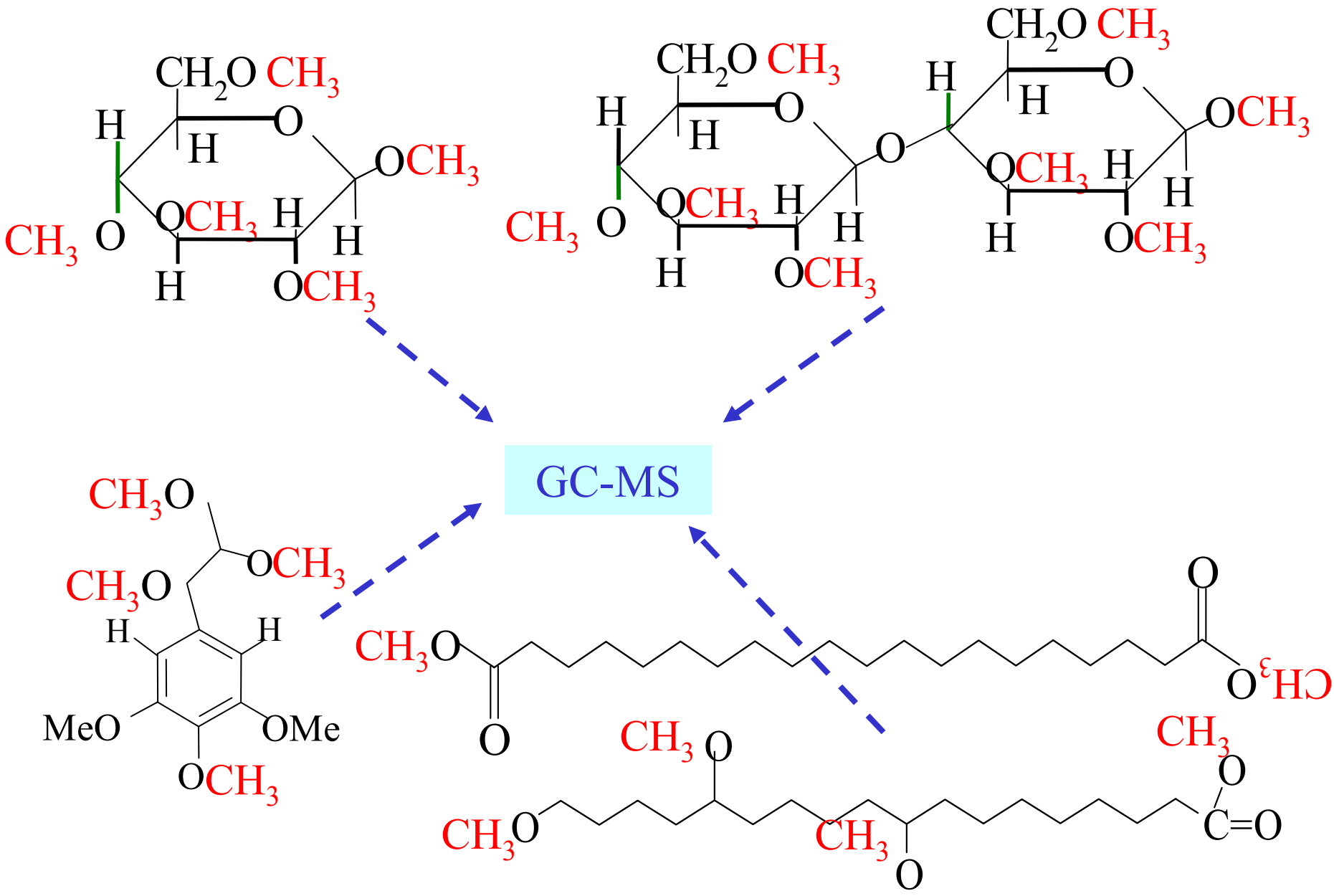
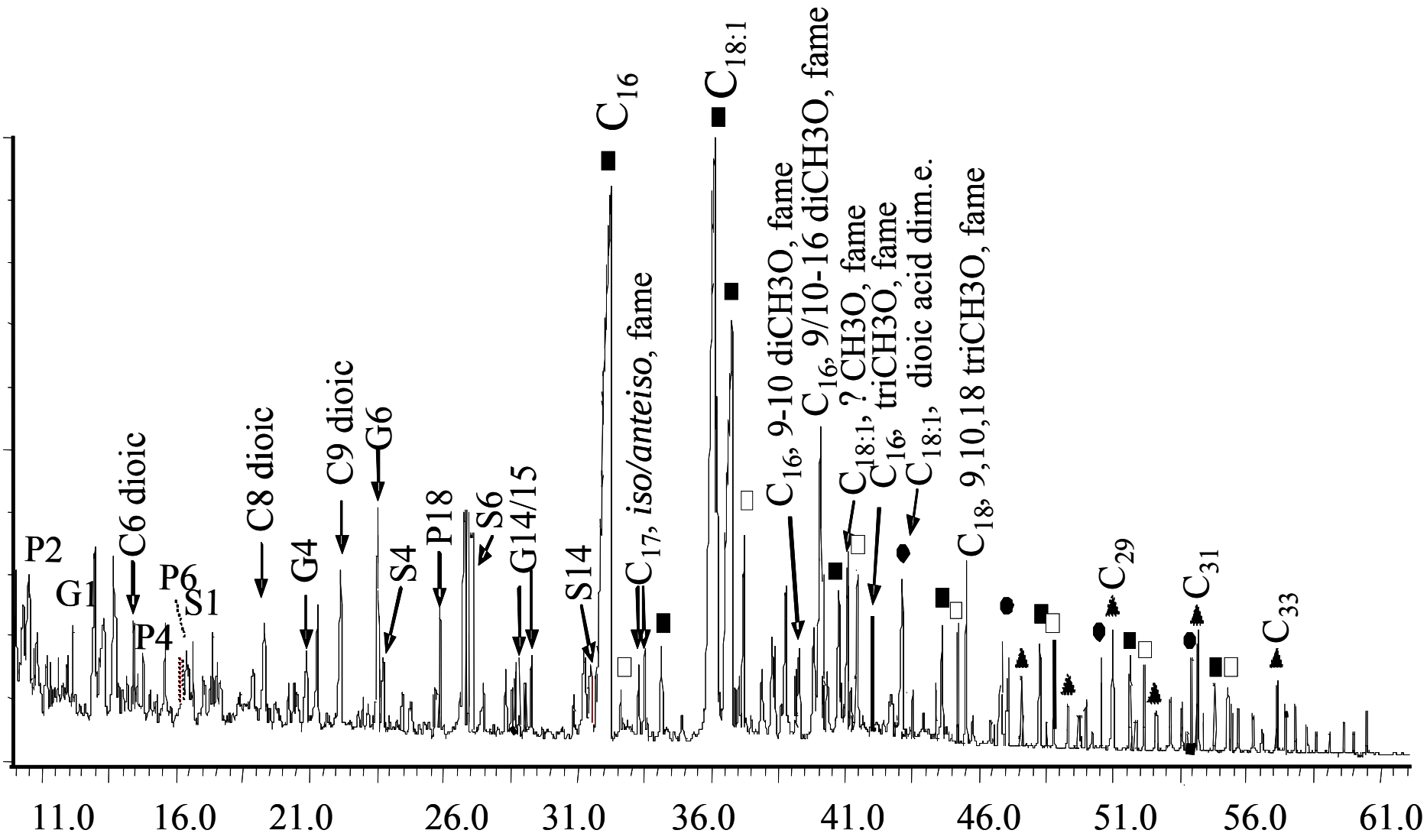
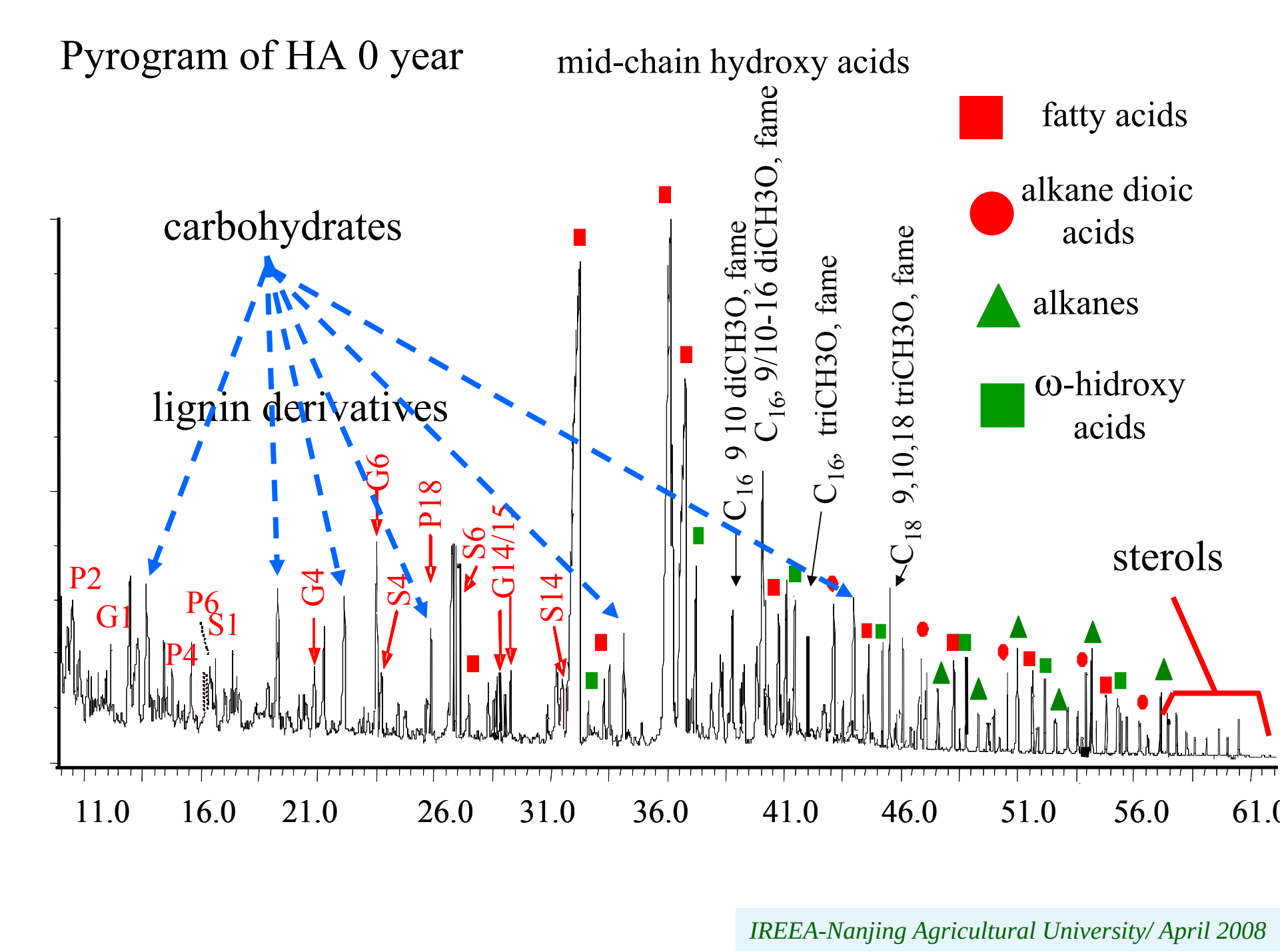

GC-MS

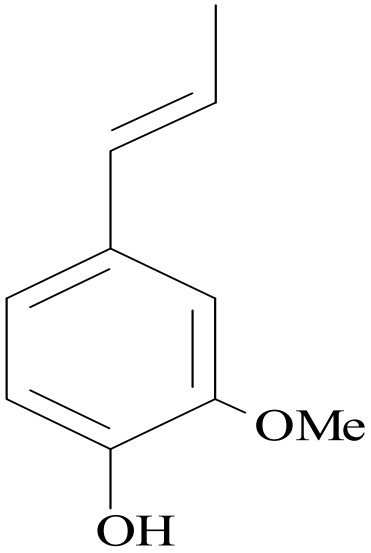
Pyrogram of HA 0 year



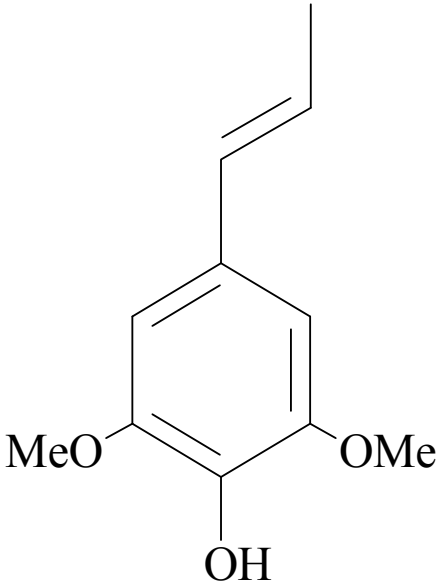

Pyrogram of HA 0 year
mid-chain hydroxy acids
fatty acids
alkane dioic acids
alkanes
ω-hidroxy acids
carbohydrates
lignin derivatives
sterols
P2
G1
P6
S1
P4
G4
G6
S4
P18
S6
G14/15
S14
C16 9 10 diCH3O, fame
C16, 9/10-16 diCH3O, fame
C16, triCH3O, fame
C18 9,10,18 triCH3O, fame
11.0
16.0
21.0
26.0
31.0
36.0
41.0
46.0
51.0
56.0
61.0

basic lignin units

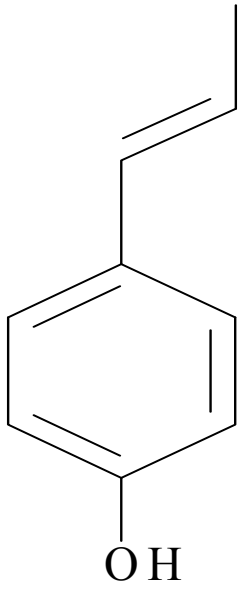
gimnosperm wood	Guaiacyl unit
angiosperm wood	Siringyl units (prevalent)+guaiacyl
erbaceous plant	P-hydroxy phenyl propene (prevalent) + guaiacyl and siringyl moieties



2-methoxy-4-propenyl phenol



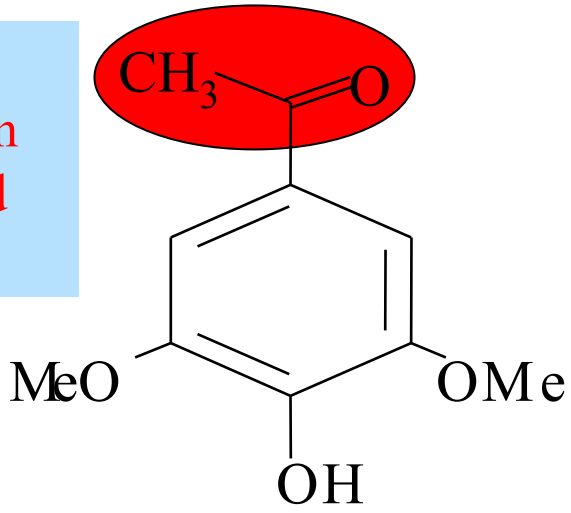
2,6-dimethoxy-4-propenyl phenol

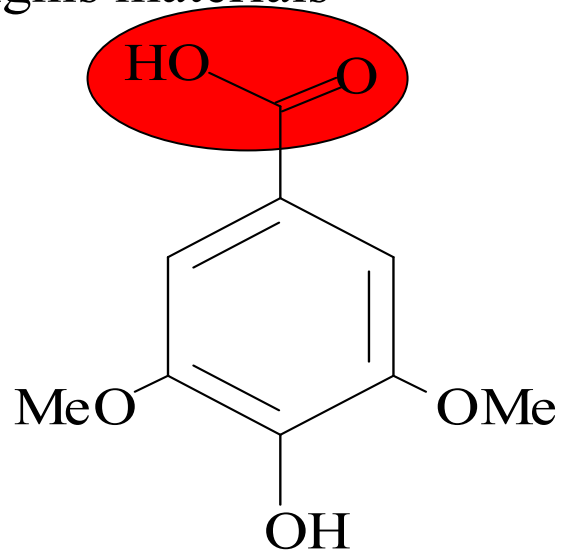


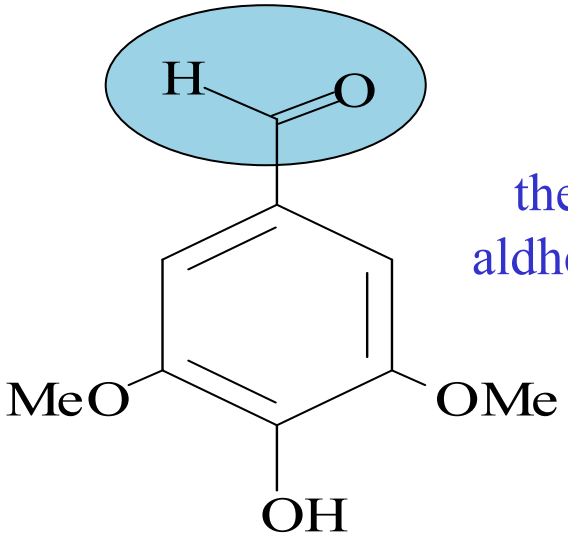
P-hydroxy phenyl propene

from the data of lignin derivatives it is possible to determine a structural index related to the degradation stage of lignis materials

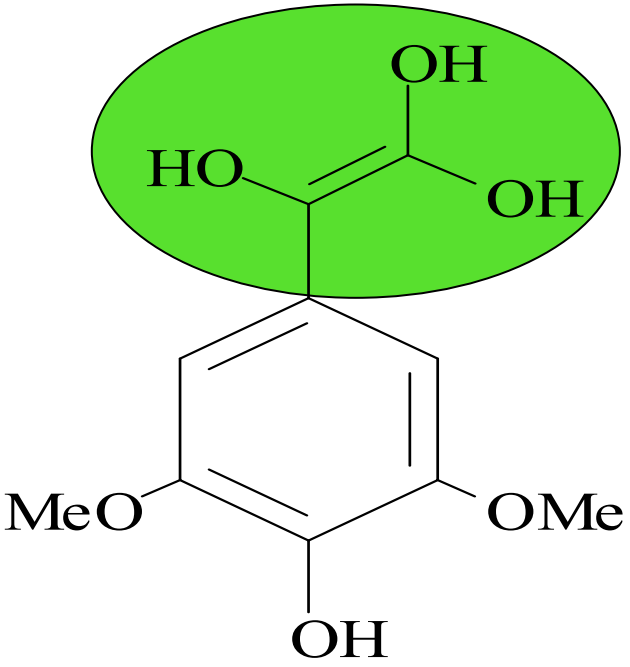
the lignin derivatives with ketonic and acidic side chain indicate in fact an advanced degradation process







the lignin derivatives with aldheidic side chain indicate a partial degradation



whereas lignin products with intact side chain are representative of unaltered or fresh plant tissues

Termochemolysis: lignin products (μg g^{-1}dry weight)

	HA 0 year
p-hydroxy-phenyl propene	2480
Guaiacyl Ad/Al_G Γ_G^c	5240 3.8 2.9

index > 2
prevalence of oxydisized structures
(high degradation)

Ad/Al=G6/G4

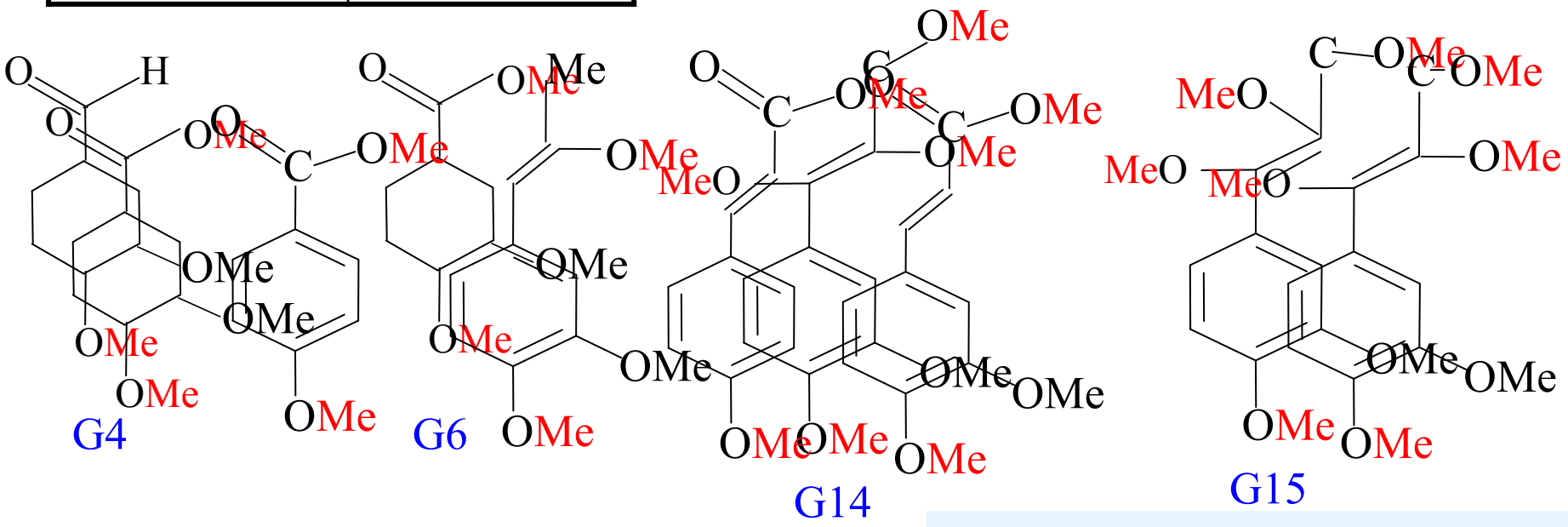
$\Gamma_G = G_6/G_{14}+G_{15}$

structural indexes

Ad=acidic form G6

Al=aldehydic form G4

Undegraded structures
G14 +G15



Termochemolysis: lignin products (μg g^{-1}dry weight)

	HA 0 year
p-idrossifenil-2-propene	2480
Guaiacyl Ad/Al_G Γ_G^c	5240 3.8 2.9
Siringyl Ad/Al_S Γ_S	4950 5.0 3.1

index > 2
prevalence of oxydisized structures
(high degradation)

Ad/Al=S6/S4

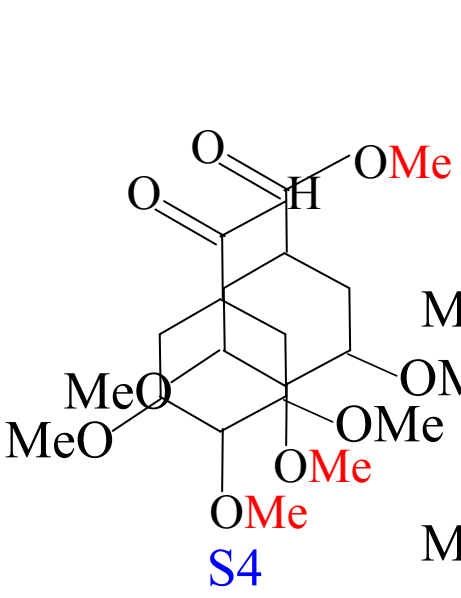
$\Gamma_S = S_6/S_{14}+S_{15}$

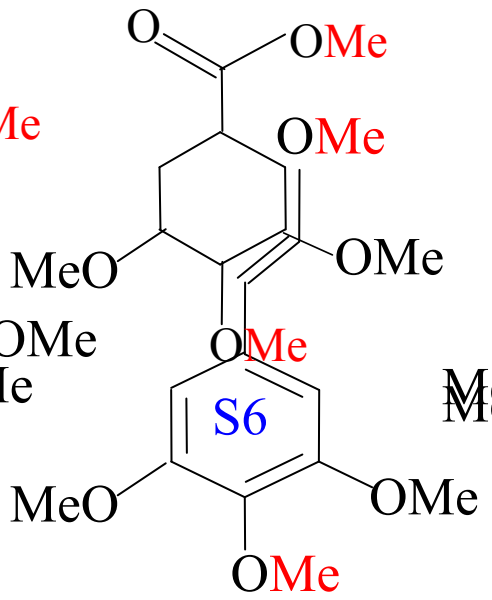
structural indexes

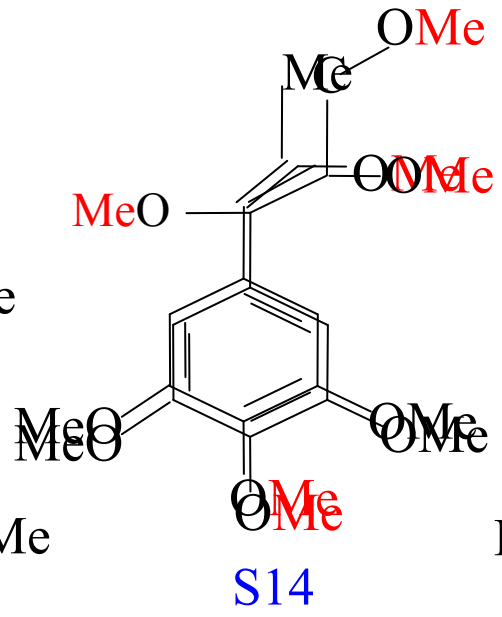
Ad=acidic form S6

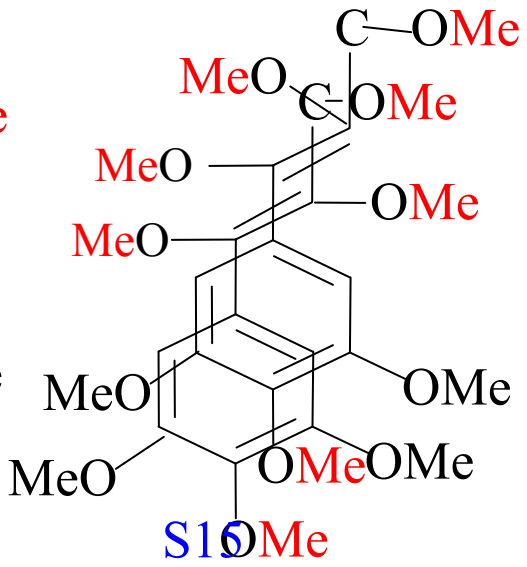
Al=aldehydic form S4

Undegraded structures
S14 +S15





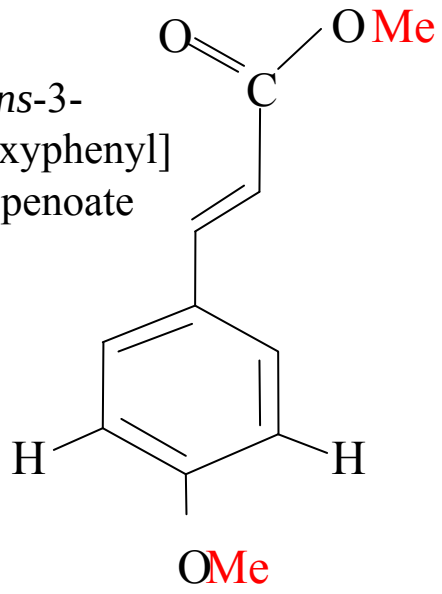




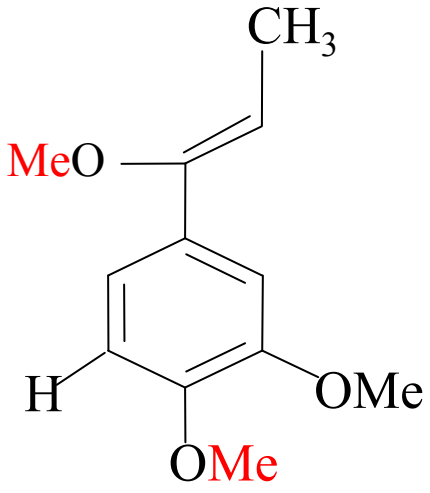
Termochemolysis of soil umic acids: lignin products (μg g^{-1} d.w.)

	HA 0 year	HA 1 year	HA 2 year
p-idrossifenil-2-propene	2480	2370	2550
Guaiacyl Ad/Al_G $\Gamma_G{}^c$	5240 3.8 3.9	5920 4.1 3.8	5830 4.3 3.0
Siringyl Ad/Al_S Γ_S	4950 5.0 4.1	5210 4.9 3.9	4730 5.1 3.6

s.d.
±15%

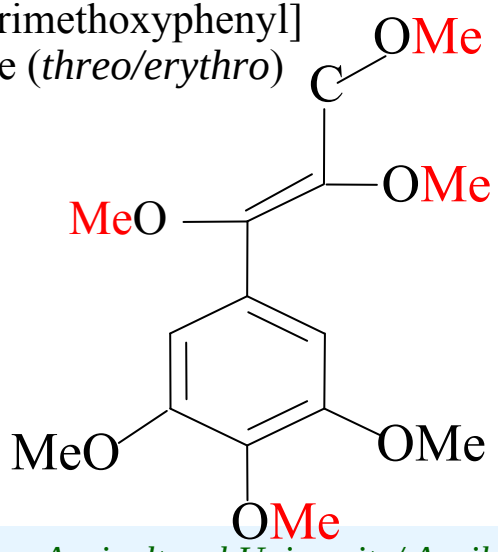


trans-3-[3methoxyphenyl]-3-propenoate



cis/trans-1-methoxy-1-(3,4-dimethoxyphenyl)-1-propene

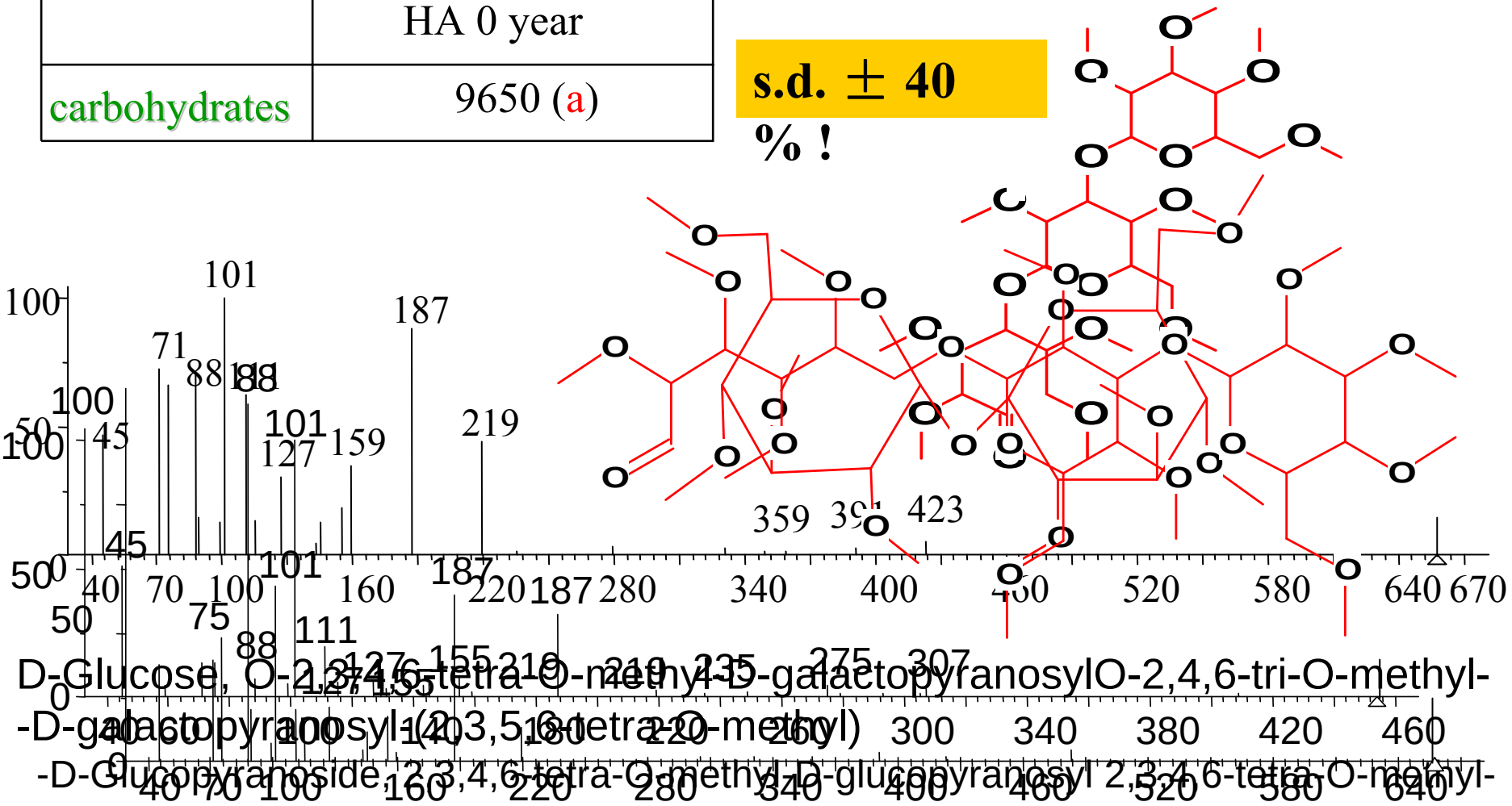
1,2,3-trimethoxy-1-[3,4,5- trimethoxyphenyl] propane (*threo/erythro*)



Termochemolysis of soil humic acids: μg g^{-1} dry weight

	HA 0 year
carbohydrates	9650 (a)

s.d. ± 40 % !



D-Glucose, O-2,3,4,6-tetra-O-methyl-D-galactopyranosylO-2,4,6-tri-O-methyl-
-D-galactopyranosyl-(2,3,5,6-tetra-O-methyl)
-D-Glucopyranoside,2,3,4,6-tetra-O-methyl-D-glucopyranosyl 2,3,4,6-tetra-O-methyl-

D-Glucose, O-2,3,4,6-tetra-O-methyl-á-D-glucopyranosyl
2,4,6-tri-O-methyl-D-glucopyranosyl2,3,4,5-tetra-O-methyl-

thermochemolysis is not well suited for the analysis of carbohydrates

the large amount of hydroxyl groups in carbohydrates and polysaccharides produce an higher sensitivity of this compounds towards the analytical conditions of thermochemolysis

the application of higher temperature for long time (30 min) and the alkaline condition provided by TMAH reagent, produce a large pyrolytic rearrangement of the hydroxyl functional groups, with loss of water molecules, cyclization and aromatization of carbohydrates molecules

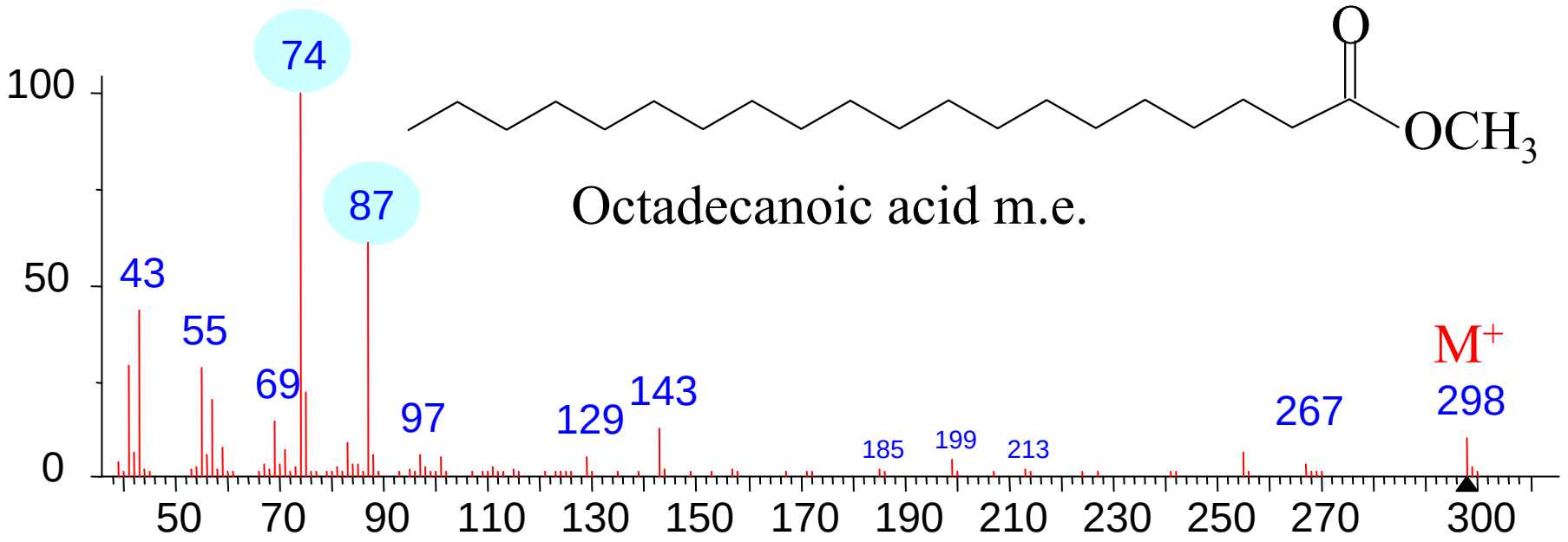
these drawbacks alter the response of polysaccharides to thermochemolysis

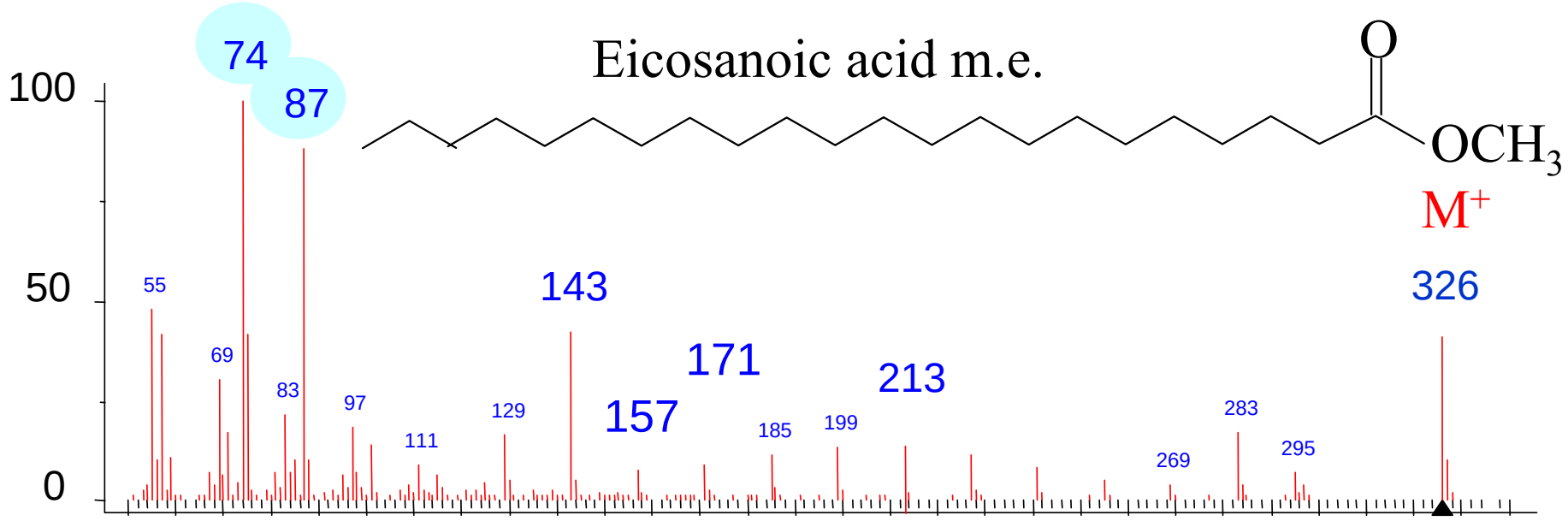
- decrease the released amount of carbohydrated products (only qualitative or semiquantitative analysis)
- lower reproducibility between replicates (higher standard errors)

Termochemolysis of soil humic acids: alkyl components (μg g^{-1} d. w.)

	HA 0 year
carbohydrates	9650 (a)
fatty acids	36100 $C_{12} \div C_{30}$(a)

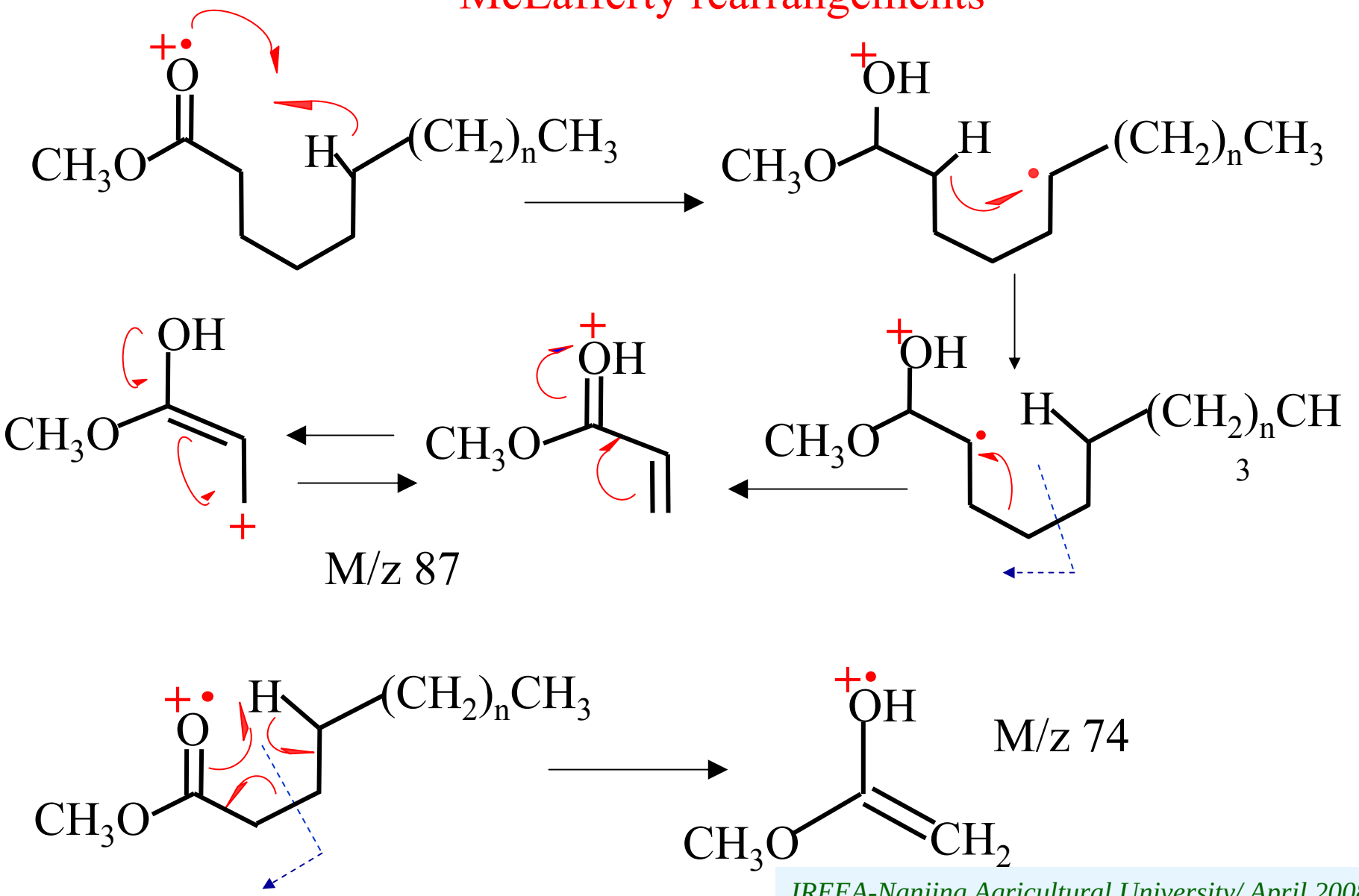
overall s.d. ± 15 %


Octadecanoic acid m.e.
O
OCH3
M+
298
74
87
43
55
69
97
129
143
185
199
213
267
100
50
0
50 70 90 110 130 150 170 190 210 230 250 270 300


Eicosanoic acid m.e.
O
OCH3
M+
326
74
87
55
69
83
97
111
129
143
157
171
185
199
213
269
283
295
100
50
0

Fragmentation of fatty acid methyl esters

McLafferty rearrangements



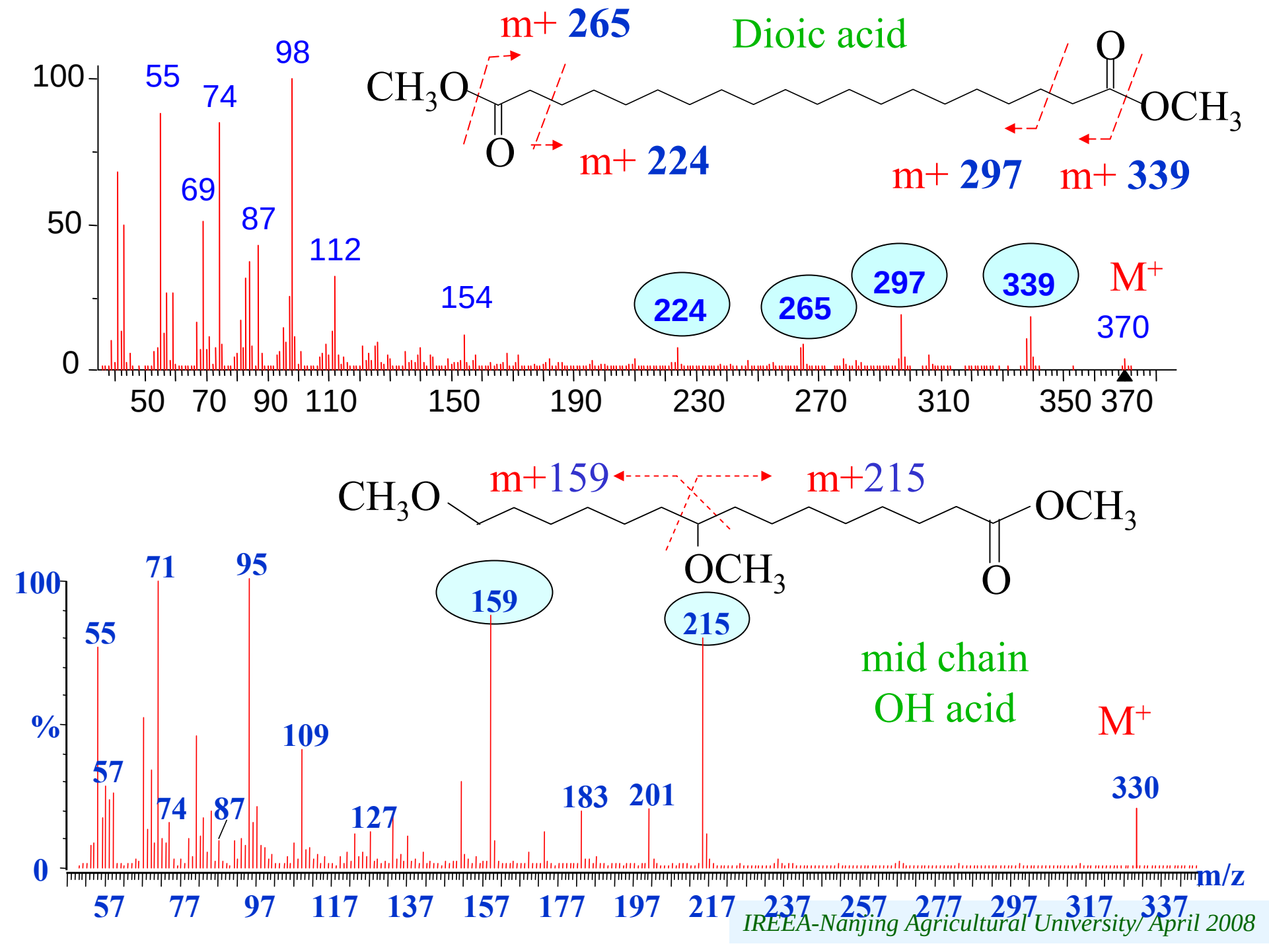
Termochemolysis of soil humic acids: alkyl components (μg g^{-1} d. w.)

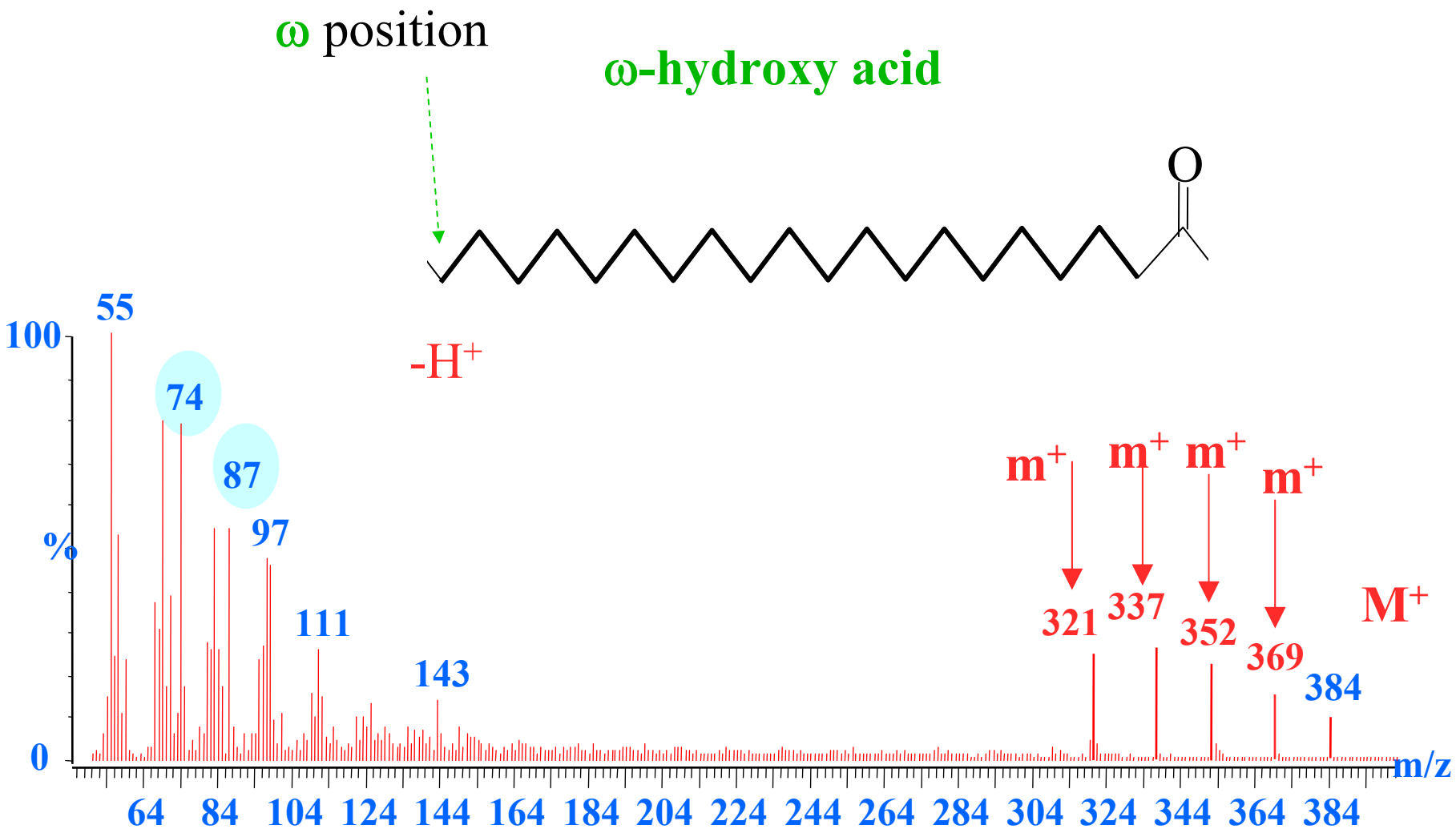
	HA 0 year
carbohydrates	9650 (a)
fatty acids	36100 C_{12}÷C_{30}(a)
ω-hydroxy acids	12850 C_{14}÷C_{26} (a)
mid-chain hydroxy acids	10350 $C_{16,}$ C_{18} (a)
alkane dioic acids	9150 $C_{18:1}$÷C_{24} (a)
α–β hydroxyacids	2100 (a) C_{12} – C_{26}

overall s.d. ± 15 %

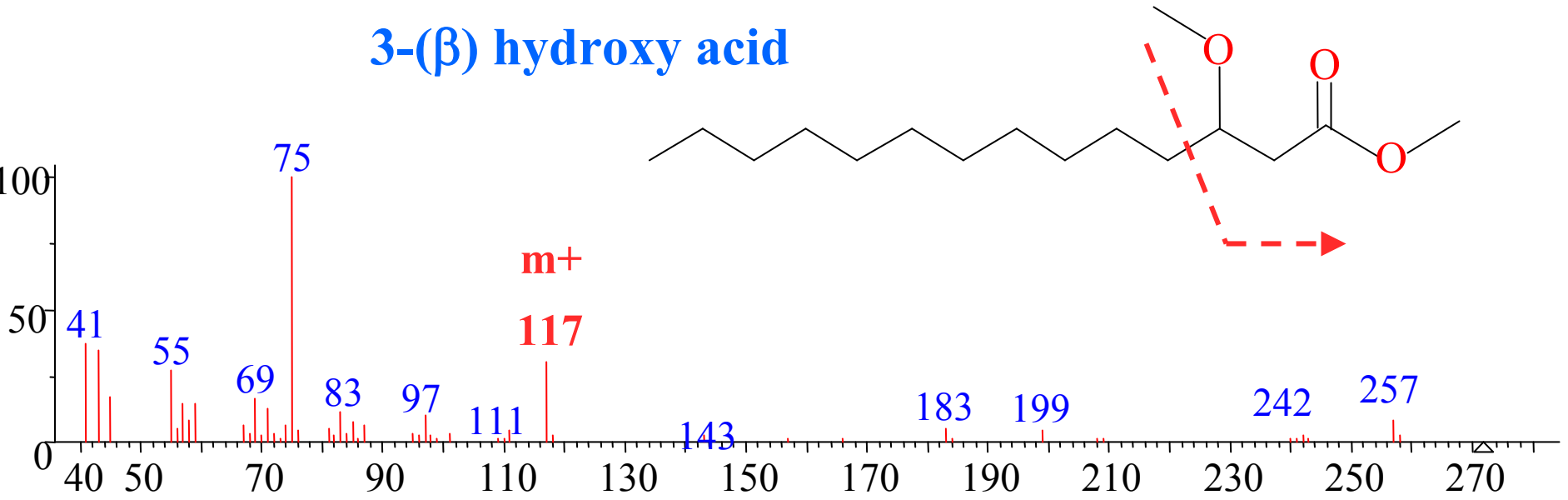
plant markers
cutin and suberin components

microbial markers

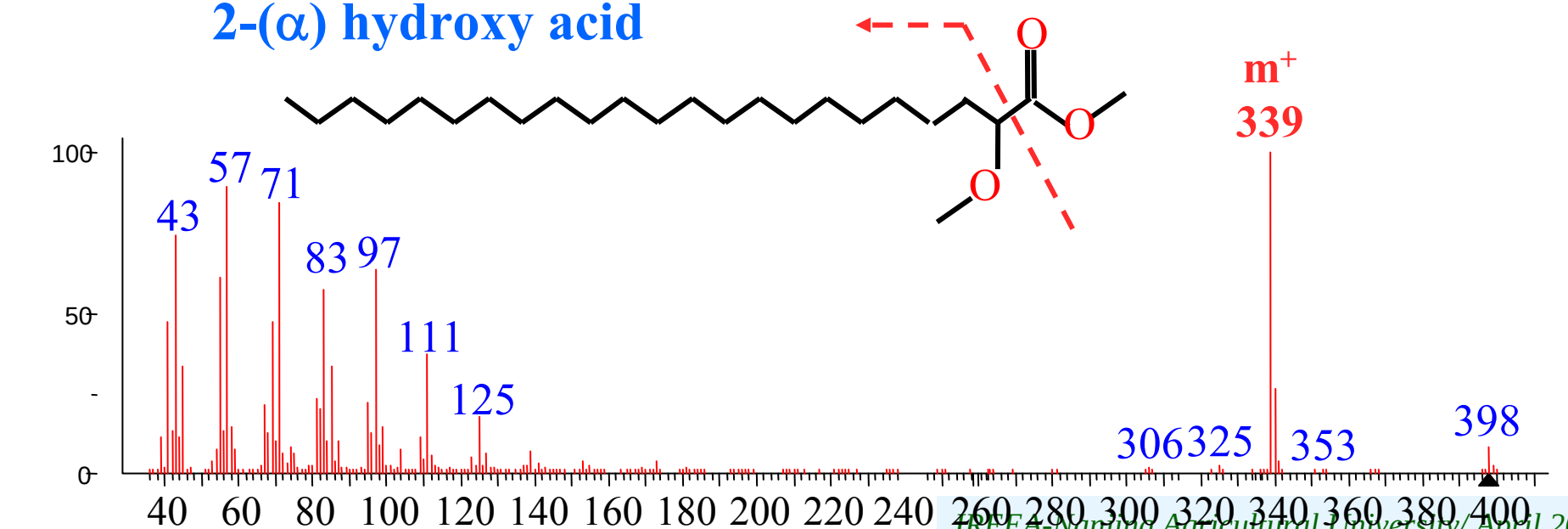

m+ 265
Dioic acid
CH3O
O
OCH3
m+ 224
m+ 297
m+ 339
100
50
0
55
74
98
69
87
112
154
224
265
297
339
M+
370
50 70 90 110 150 190 230 270 310 350 370
m+159
m+215
CH3O
OCH3
OCH3
O
100
%
0
71
95
55
159
215
109
57
74
87
127
183
201
330
mid chain
OH acid
M+
m/z
57 77 97 117 137 157 177 197 217 237 257 277 297 317 337


ω position
ω-hydroxy acid
O
100
%
0
55
74
87
97
111
143
-H+
m+
m+
m+
m+
321
337
352
369
384
M+
m/z
64
84
104
124
144
164
184
204
224
244
264
284
304
324
344
364
384

3-(β) hydroxy acid



2-(α) hydroxy acid



Termochemolysis of soil humic acids: alkyl components (μg g^{-1} d. w.)

	HA 0 year
carbohydrates	9650 (a)
fatty acids	36100 C_{12}÷C_{30}(a)
ω-hydroxy acids	12850 C_{14}÷C_{26} (a)
mid-chain hydroxy acids	10350 $C_{16,}$ C_{18} (a)
alkane dioic acids	9150 $C_{18:1}$÷C_{24} (a)
α–β hydroxyacids	2100 (a) C_{12} – C_{26}
alkanes	6140C_{25}÷C_{33} (a)
sterols	3200 (a)
diterpenoids	2230 (a)

overall s.d. ± 15 %

plant markers
cutin and suberin components

microbial markers

plant markers
angiosperm
gimnosperm

Termochemolysis of soil humic acids: μg g^{-1} dry weight

	HA 0 year	HA 1 year	HA 2 year
carbohydrates	9650 (a)	8700 (a)	8730 (a)
fatty acids	36100 C_{12}÷C_{30}(a)	15100 C_{12}÷C_{30} (b)	14200 C_{12}÷C_{30} (b)
ω-hydroxy acids	12850 C_{14}÷C_{26} (a)	9150 C_{14}÷C_{26} (b)	9300 C_{14}÷C_{26} (b)
mid-chain hydroxy acids	10350 $C_{16,}$ C_{18} (a)	8780 $C_{16,}$ C_{18} (b)	9200 $C_{16,}$ C_{18} (b)
alkane dioic acids	9150 $C_{18:1}$÷C_{24} (a)	6300 $C_{18:1}$÷C_{24} (b)	5900 $C_{18:1}$÷C_{24} (b)
α–β hydroxyacids	2100 (a) C_{12} – C_{26}	1900 (b) C_{12} – C_{26}	2300 (b) C_{12} – C_{26}
alkanes	6140C_{25}÷C_{33} (a)	3790 C_{25}÷C_{33} (b)	3900 C_{25}÷C_{33} (b)
sterols	3200 (a)	3210 (a)	3140 (a)
diterpenoids	2230 (a)	2300 (a)	2250 (a)

- TMAH-thermochemolysis technique is a rapid and effective method to obtain direct qualitative and quantitative evaluation of complex organic materials like humic substances - thermochemolysis released more than hundred different molecules; plant biopolymers like lignin, waxes and aliphatic polyesters were recognized as the main sources of humic acids
- Lipids, lignin, and carbohydrates were the main components of soil HA extracted after organic matter addition - the main variations were represented by a large decrease of bio-available lipids components such as fatty acids and linear hydrocarbon
- Lower decrease and large persistence were found for biopolyester and lignin components – these findings confirm previous results on the formation of Humic substances through the selective accumulation of these recalcitrant organic molecules

Advantages and drawbacks of thermochemolysis

- quick sample preparation and analysis
  1 sample (3 replicates) 1 day
- simultaneous characterization of various organic components (lignin, lipids, biopolyesters, carbohydrates)
- thermochemolysis products are ready for GC-MS analysis without any pre-treatment (like purification andderivatization)
- slow interpretation of complex chromatograms
- lower reproducibility: high standard deviation
- about 50% of TOC is not released: semi-quantitative evaluation

Thanks for
your attention