12.158 Lecture 1

Mass Spectrometry

Some Fundamental Background Information for Organic Geochemists and Geobiologists

Reference:

McLafferty & Tureček

Interpretation of Mass Spectra 4th Ed

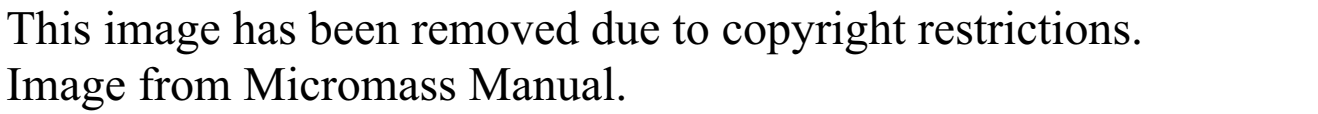
http://i-mass.com/

Contents

- **Mass Spectrometers and their component parts, things you need to know**
- **Formation of mass spectrum**
- **Interpretation of mass spectra**
- **Detection of specific compounds using ion chromatograms**
- **Selected ion recording (SIR)**
- **Multiple Reaction Monitoring (MRM and MRMQ)**
- **LC-MS**

Components of the Mass Spectrometer (1)

- **Vacuum System**
  - **any collisions of an ion with a gas molecules in its path destroys it --> no MS**
- **System to convert intact molecules to ions -**
  THE ION SOURCE
  - **electron impact (EI), chemical ionisation (volatiles)**
  - **electrospray or sputtering (non-volatiles eg proteins, DNA)**
- **Mass Analyser - To filter by mass (and KE)**
- **Ion Detector and ion current amplifier**
- **Display mechanisms and Data Acquisition System**



AUTOSPEC Mass Spectrometer

Example: Vacuum System Schematic for Double Focussing MS VG 70E

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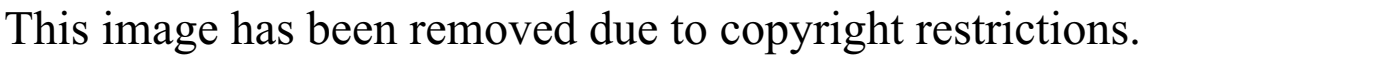
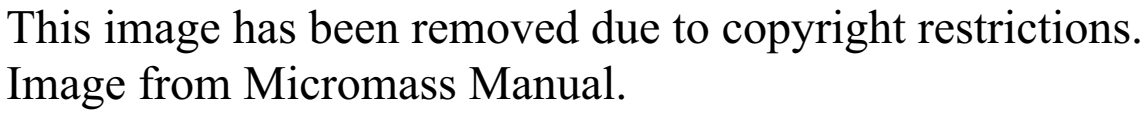
Image from Micromass Manual.

Source Options: Micromass Autospec



Outer: lenses and heater



Inner: ion chamber, filament, repeller; easily cleaned/swapped

Components of the Mass Spectrometer (2)

- **Mass Analyser - To filter by mass (and KE)**
  - **single magnet or quadrupole --> low resolution ~ 1 dalton**
  - **double sector- magnet plus electrostatic analyser --> 'high resolution' --> accurate mass to 1-2ppm --> elemental composition by mass defect**
  - **multiple sector --> hybrid such as Autospec Q or triple quadrupole (TSQ) --> target compound analysis by reaction chromatography**
  - **time of flight (TOF), ion cyclotron resonance**
- **Ion Detector and ion current amplifier**
- **Display mechanisms and Data Acquisition System**

Components of the Vacuum System

- 1st Stage --> Rotary pump(s)
  - monitored by Pirani guage (pump to 10^{-2} torr)

    problems - oil level, bearings, drive, dirty oil
- 2nd Stage --> Oil Diffusion pump(s)
  - monitored by ion guage (pumps to 10^{-8} torr)
  - 70E and Autospec are 'differentially pumped' to give better vacuum in the analyser and hence better resolution

    problems - oil level, cooling water, vacuum interlocks
- Isolation valves to separate pumps, source, analyser

problem - interlocks

- 2nd Stage can be turbomolecular pump(s) (pumps to 10^{-9} torr)

Components of the Ion Source

- **Ion volume (or block) – Autospec accelerating potential 6-8kV**
  - **space to form the ions** problems - dirt, high voltage
- **Filament**
  - **hot tungsten wire creates an electron beam**

  problems - broken, misaligned, bad contacts, low emission
- **Repeller and focus places**
  - **causes ions to exit source as collimated beam**

  problems - bad contacts, ion burns
- **Exit slit**
  - **causes ion beam to enter analyser with tightly defined dimensions** problems - ion burns, slide mechanism

Components of the Analyser

- **Electrostatic analyser (2 for Autospec)**
  - **Filters ions for kinetic energy. Narrow energy window allows magnet to gives less dispersion and better separation**
- **Magnetic analyser**
  - **Electromagnet driven by a high current**

    problems - current control, cooling water, interlocks
- **Collision cells - fill with gas to cause fragmentation**
- **Collector slit**
  - **causes ion beam to enter detector with tightly defined dimensions**

Components of the Mass Spectrometer (3)

- **Ion Detector (must be fast response)**
  - **Off axis detectors so need deflector plate to bend beam**
  - **Electron multiplier (HP) continuous or discrete dynode**
  - **Photomultiplier (Autospec)**
  - **Preamplifier and amplifier --> gain and noise controls**

  problems - slow degradation, noise

- **System for Display and Acquisition**
  - **Analogue to digital conversion noise filtering and mass measurement**
  - **data storage, manipulation and presentation**

    computer bugs, software evolution

Separate physical and electronic bits

- **Physical bits need to be clean**
  - **most problems come down to dirt of some kind or other**
  - **most problems occur in the GC or in the source**

- **Develop skills to distinguish dirt and other contamination from electronic faults which are much harder to resolve**

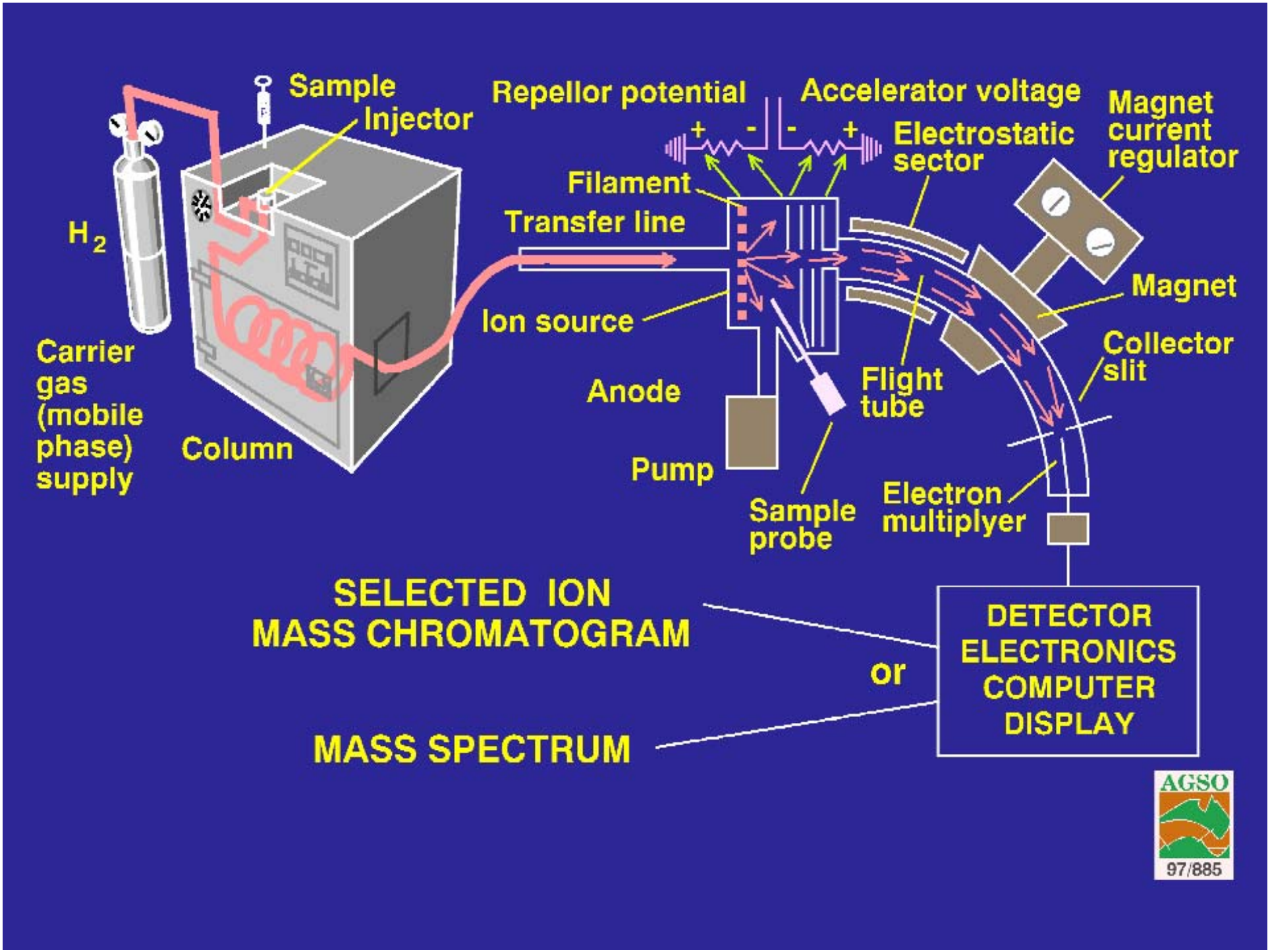
Inlet Systems

- Compound must be volatile for EI and CI
- Gas Reservoir (Hot Inlet System)
- Solids Probe
- Gas Chromatograph
- Liquid Chromatograph

Ionization Methods

- Electron Impact Ionization
  - High energy, fragmentation = information
- Chemical Ionization
  - Low energy collisions with a gas such as CH4, NH3

- Electrospray LC-MS
- Atmospheric Pressure CI LCMS


Sample
Injector
Repellor potential
Accelerator voltage
Magnet
current
regulator
Electrostatic
sector
Filament
Transfer line
H_2
Magnet
Ion source
Collector
slit
Carrier
gas
(mobile
phase)
supply
Anode
Flight
tube
Column
Pump
Sample
probe
Electron
multiplyer
SELECTED ION
MASS CHROMATOGRAM
or
MASS SPECTRUM
DETECTOR
ELECTRONICS
COMPUTER
DISPLAY
AGSO
97/885

Formation of the mass spectrum

- Compound ionises in source
- Molecular ions fragments as a result of excess energy imparted by the ionising electrons (standard energy 70eV)
- Ions pushed from source with repeller
- Accelerated by high voltage
- Ions separated by mass analyser
- Ions counted and recorded

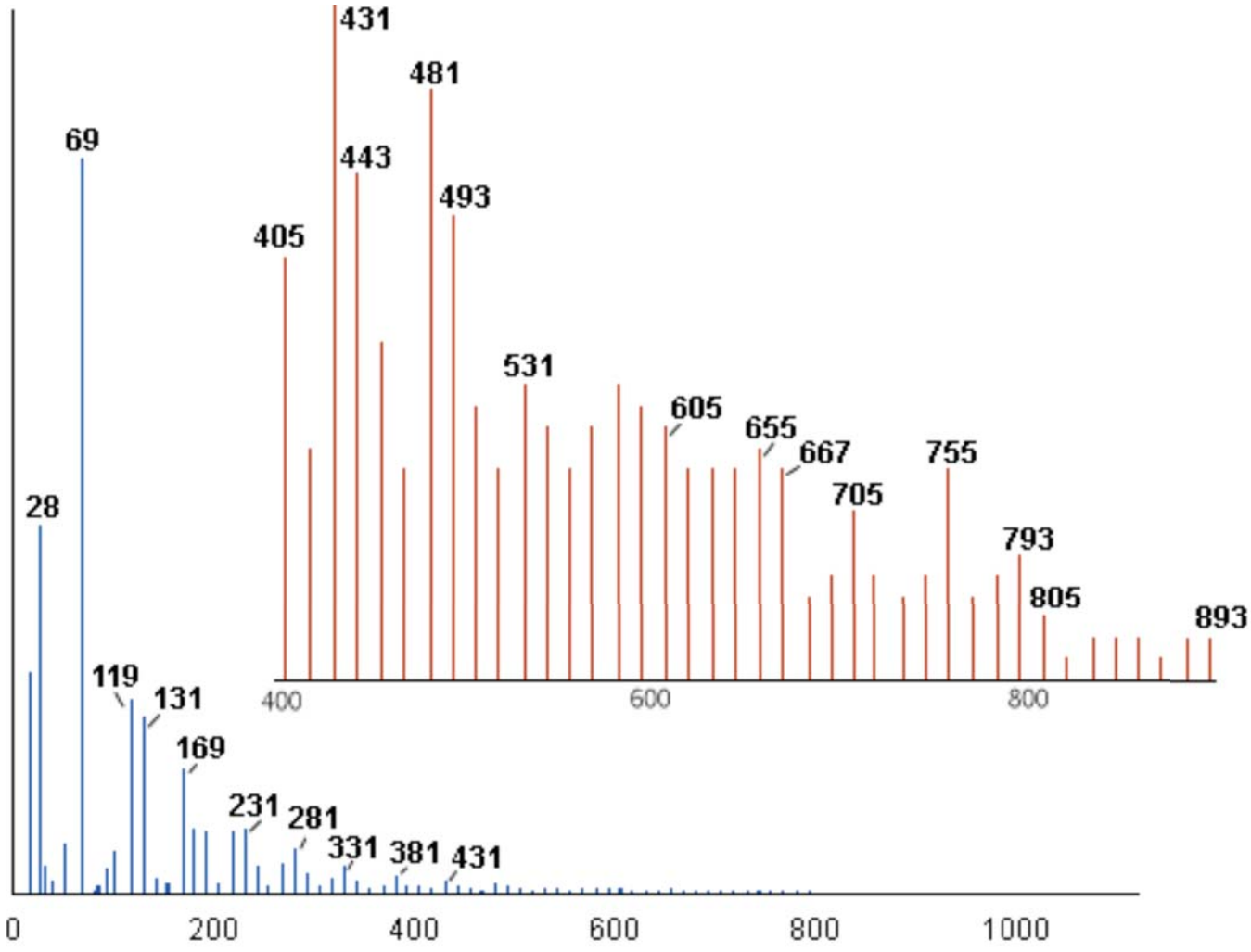
Calibration and leak checking

- **Perfluorokerosene (PFK) - a mixture of fluoroalkanes with ions to > 900 dalton**

  69, 100, 119, 219, 231 ……..

- **Heptacosafluorotributylamine - a single compound MW 614 with 69, 119, 219 ...**
- **Background spectrum should look like air;**
- **17,18,28-32 and 40 Da**
- **Argon useful to detect leaks**

Perfluorokerosene (PFK) - a mixture of fluoroalkanes with ions to > 900 dalton



m/z	Rel. Abundance	Formula	Exact Mass
18.01	30.00		
28.01	50.00		
31.0	3.80	CF	30.99840
39.96	1.70		
51.00	6.70		
69.00	100.00	C1F3	68.99521
81.00	0.50	C2F3	80.99521
84.97	1.00		
93.00	3.30	C3F3	92.99521
99.99	5.60	C2F4	99.99361
118.99	26.40	C2F5	118.99201
130.99	24.00	C3F5	130.99

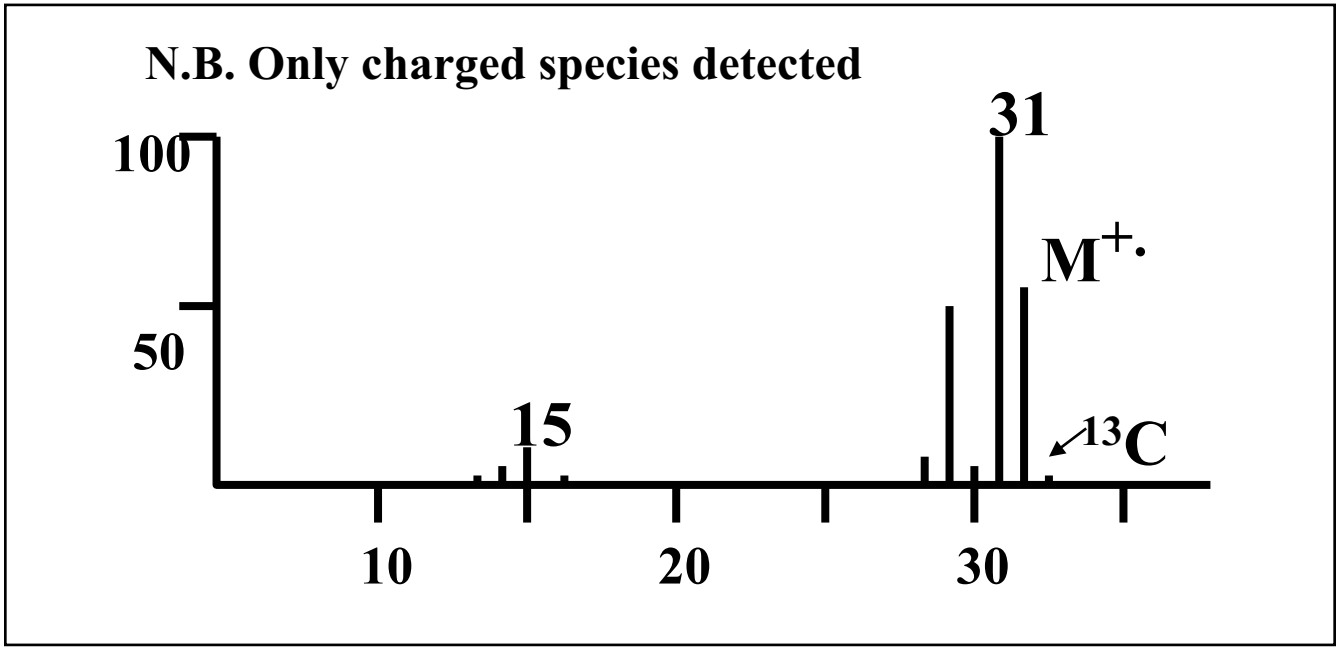
Ionisation & Fragmentation Processes

$CH_3OH + e^- \rightarrow CH_3OH^{+\cdot}$ (m/z 32) + $2\ e^-$ ionisation

$CH_3OH^{+\cdot} \rightarrow CH_2OH^+$ (m/z 31) + $H^\cdot$ sigma cleavage

$CH_3OH^{+\cdot} \rightarrow CH_3^+$ (m/z 15) + $^\cdot OH$ sigma cleavage

$CH_2OH^+ \rightarrow CHO^+$ (m/z 29) + H_2 neutral loss



Characteristic ion series

- **Silicone bleed (silicon isotopes) 207, 281, 355**
- **Alkanes 57, 71, 85, 99, 113 ...**
- **Alkenes 55, 69, 83, 97, 111 …**
- **Cyclohexanes 83, 97, 111**
- **Terpanes 123, 149, 191, 205, 217, 231**
- **Monoaromatics 77, 91, 105, 119, 133 …..**

Ionisation & Fragmentation Processes

$CH_3OH + e^- \rightarrow CH_3OH^{+\cdot}$ (m/z 32) + $2\ e^-$ ionisation

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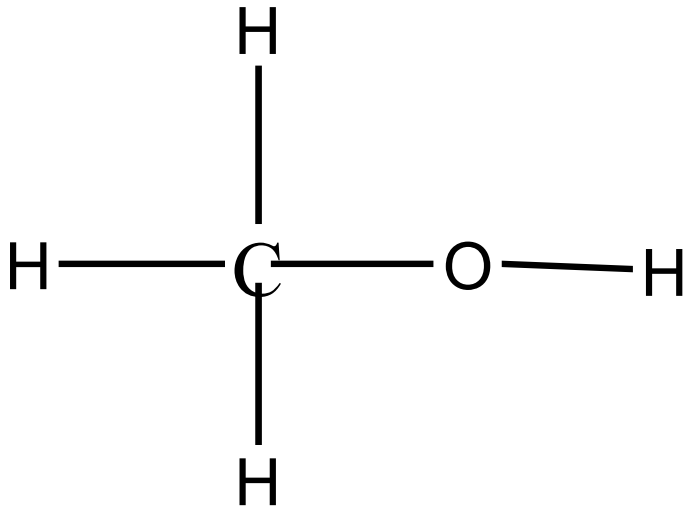


Appearance of the Mass Spectrum

- Average spectrum has 2^{50} bits of information
- Molecular ion and its isotopic abundances
- Ionisation energy and structure determine degree of fragmentation; 70 eV is standard
- Fragment abundances reflect to relative stabilities of the fragment ions - most abundant are the most stable
- Multiply charged and metastable ions lost in data reduction

Interpretation of the mass spectrum

- **Identify molecular ion**
- **Assess isotope abundances for:**
  - **A elements H and F**
  - **A+1 elements C, N**
  - **A+2 elements Si, S, Cl, Br**
- **Determine # rings and double bonds**

Acyclic saturated hydrocarbons C_nH_{2n+2}

- **Identify 'characteristic' ions and low mass series**

Interpretation of the mass spectrum

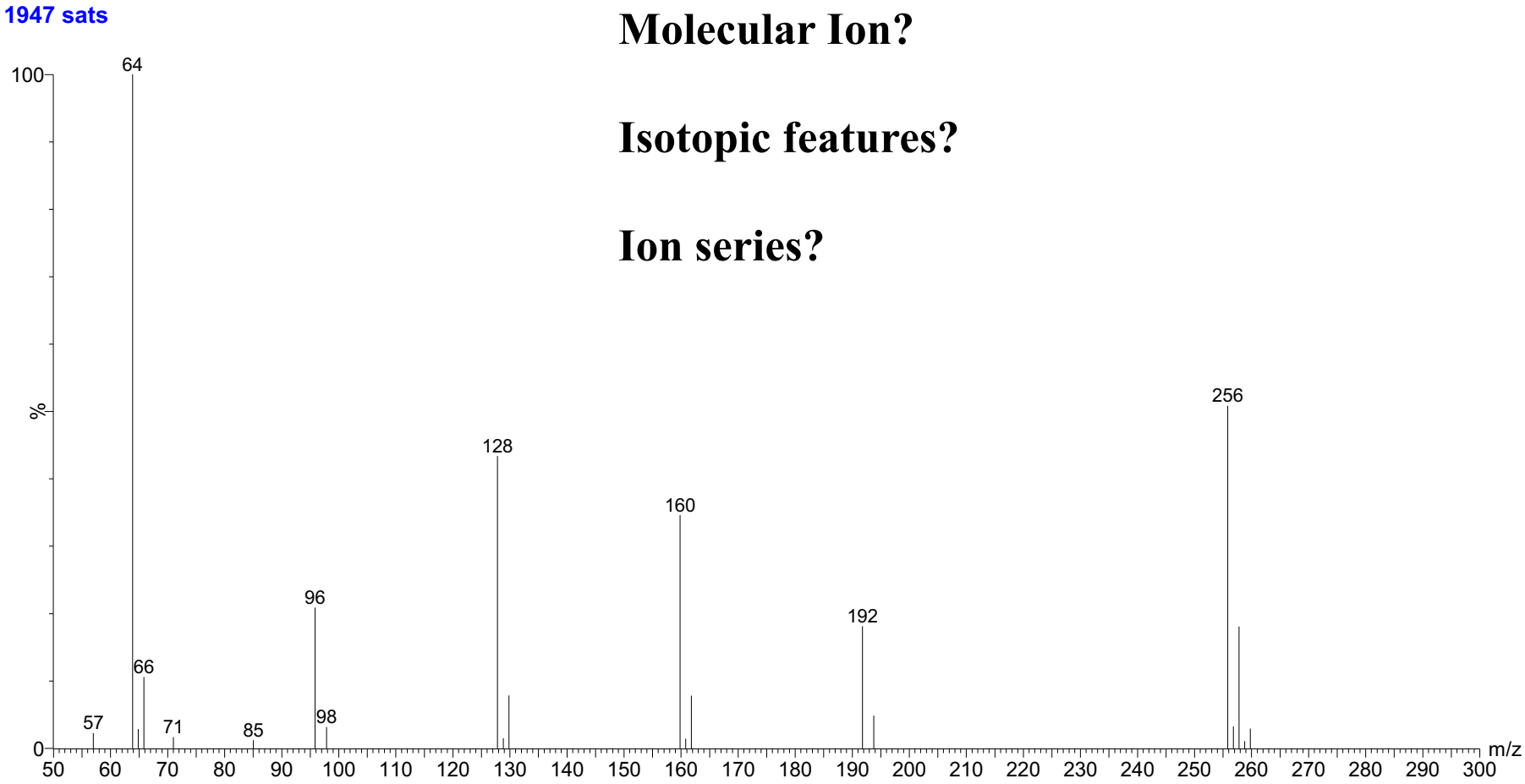
- **Identify molecular ion**
  - **Molecular Ion corresponds to the loss of one electron from the intact molecule**
  - **Molecular ion is an ‘odd electron’ ion**
  - **Odd electron ions are generally present and have an even mass unless an odd number of nitrogens**

Identify:

Molecular Ion?

Isotopic features?

Ion series?



Nuclidic Masses and Abundances

Element	Mass	Isotope Abundance
carbon	12.00000	98.9
	13.0034	1.1
hydrogen	1.007825	99.985
	2.0140	0.015
nitrogen	14.00307	99.63
	15.0001	0.37
oxygen	15.9949	99.8
	17.9992	0.2
fluorine	18.9984	100
sulfur	31.9720	94.8
	32.9715	0.8
	33.9679	4.4

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Please see Table 2.1
©McLafferty & Tureček: Interpretation of Mass Spectra 4th Ed

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Please see Table 2.2
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Please see Table 2.3
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GC-MS

Commonly done on low resolution

(1 dalton) quadrupole instruments in the selected ion (mass) mode.

e.g. Hewlett-Packard MSD, Thermo, Shimadzu

Can be done at high resolution; accurate mass

What has to be right for successful GC-MS? Every single time you try!!

•GC working with correct column in good condition, right gas flows, autosampler working, correct conditions of injection, temp program, hot interface, no leaks, no bleed, clean injector, clean syringe, clean wash solvents, correct vials and septa ……...

•Good vacuum, clean source, right source temp and ionising conditions, appropriate peak resolution & tune, correct slits, correct gain setting, low instrument noise ……..

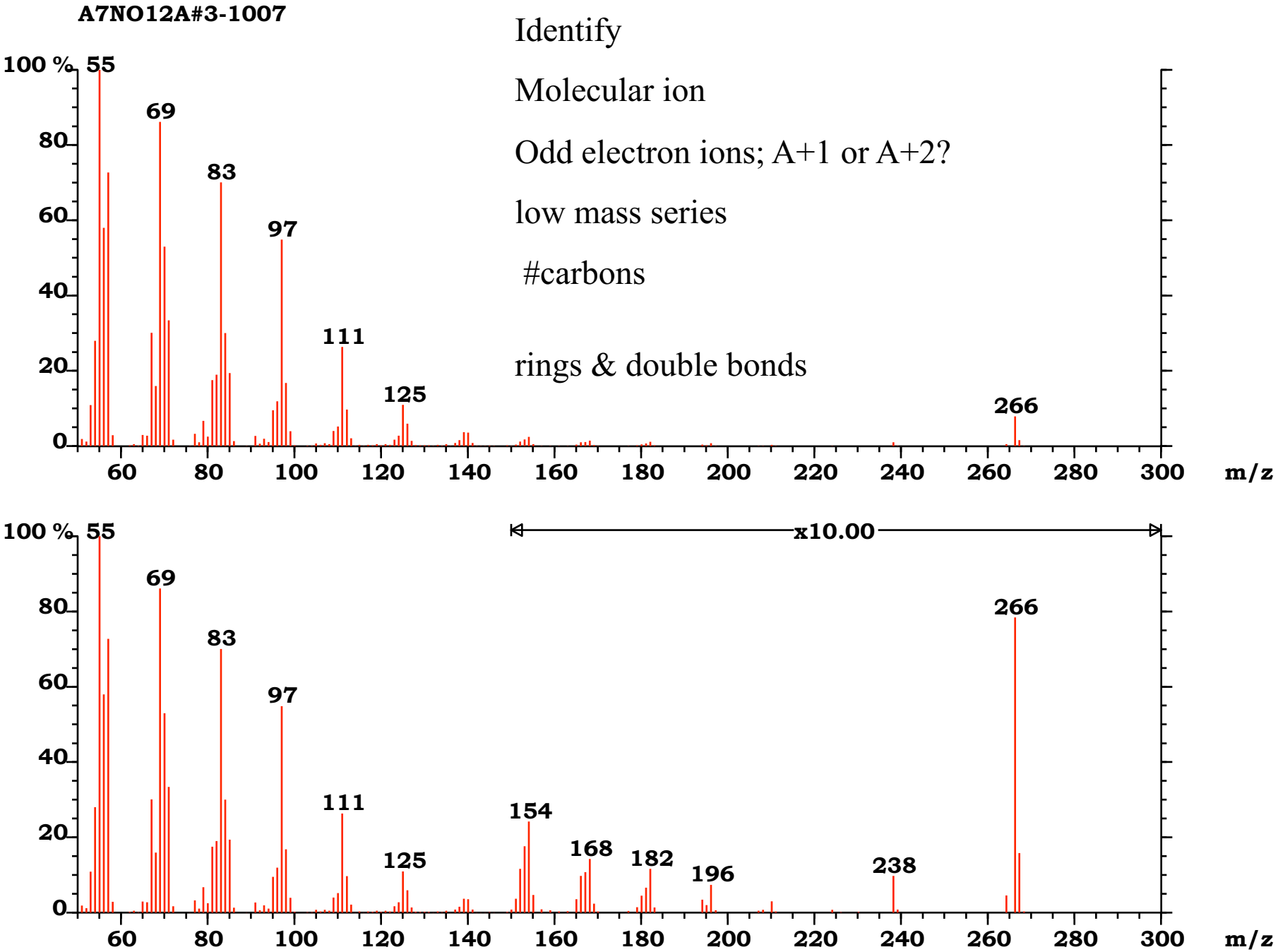
• DS interface (noise, threshold) set correctly, correct experiment for the job, instrument in calibration, file structure correct, adequate storage space, no bugs before starting sequence ……

•Samples correctly ordered in sampler, blanks & external standards included, data archived , QUAN set up correctly ….

•Interpretive skill ….

Familiarity with the instruction manual

- **Don't even think about operating a mass spectrometer without being familiar with the manual!**
- **If it is not clear, ask or read on!**
- **Be familiar with the emergency shutdown procedures**
- **Be familiar with OH&S issues (eg High Voltages)**
- **Mistakes are inevitable and part of the scenery**
  - **tell your colleagues immediately and log it so that they can be avoided by others**
  - **get help or do what you can to fix the problem**
  - **collegiality is critical when machines are shared**



Identify

Molecular ion

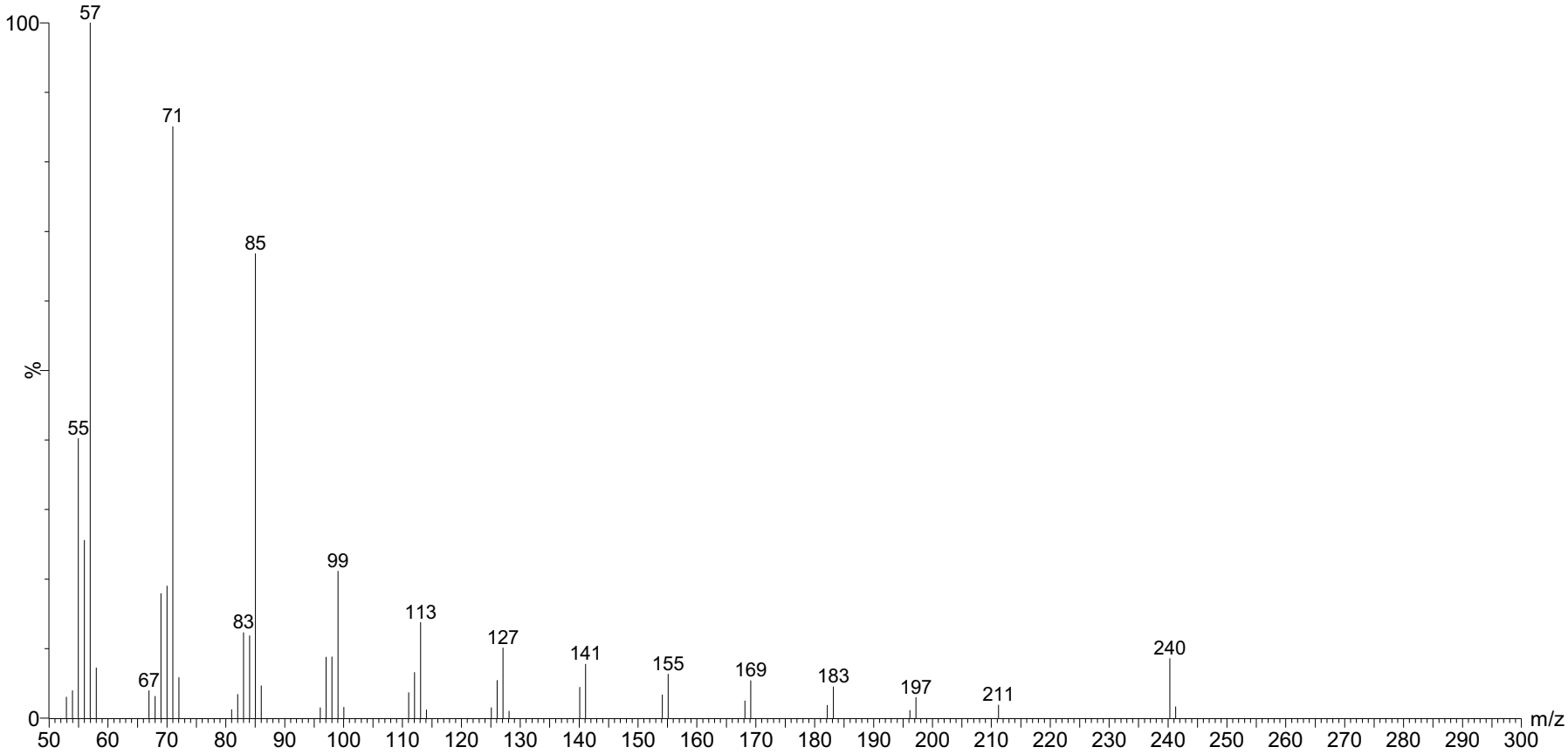
Odd electron ions; A+1 or A+2?

low mass series

#carbons

rings & double bonds

1947 sats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m/z
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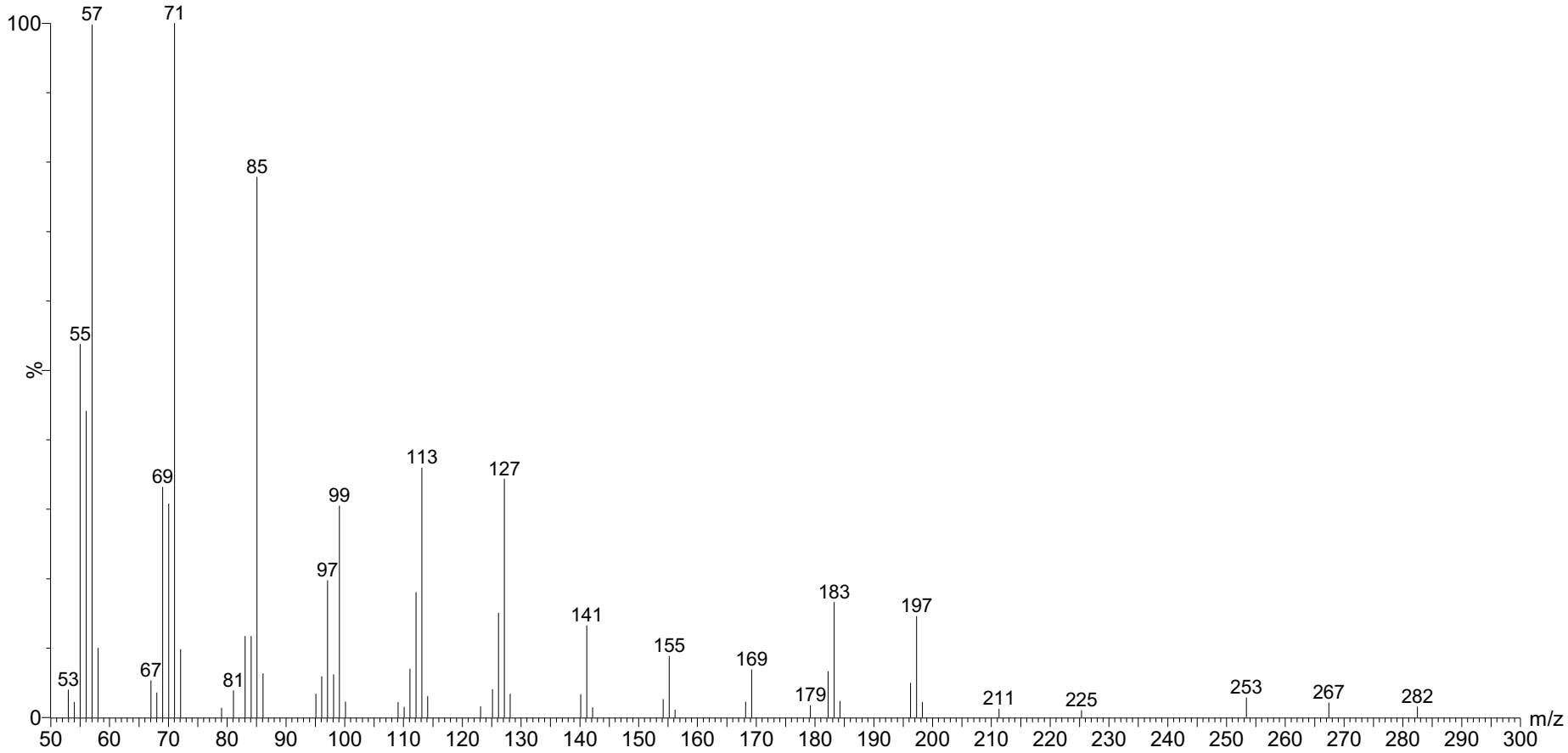
68 sats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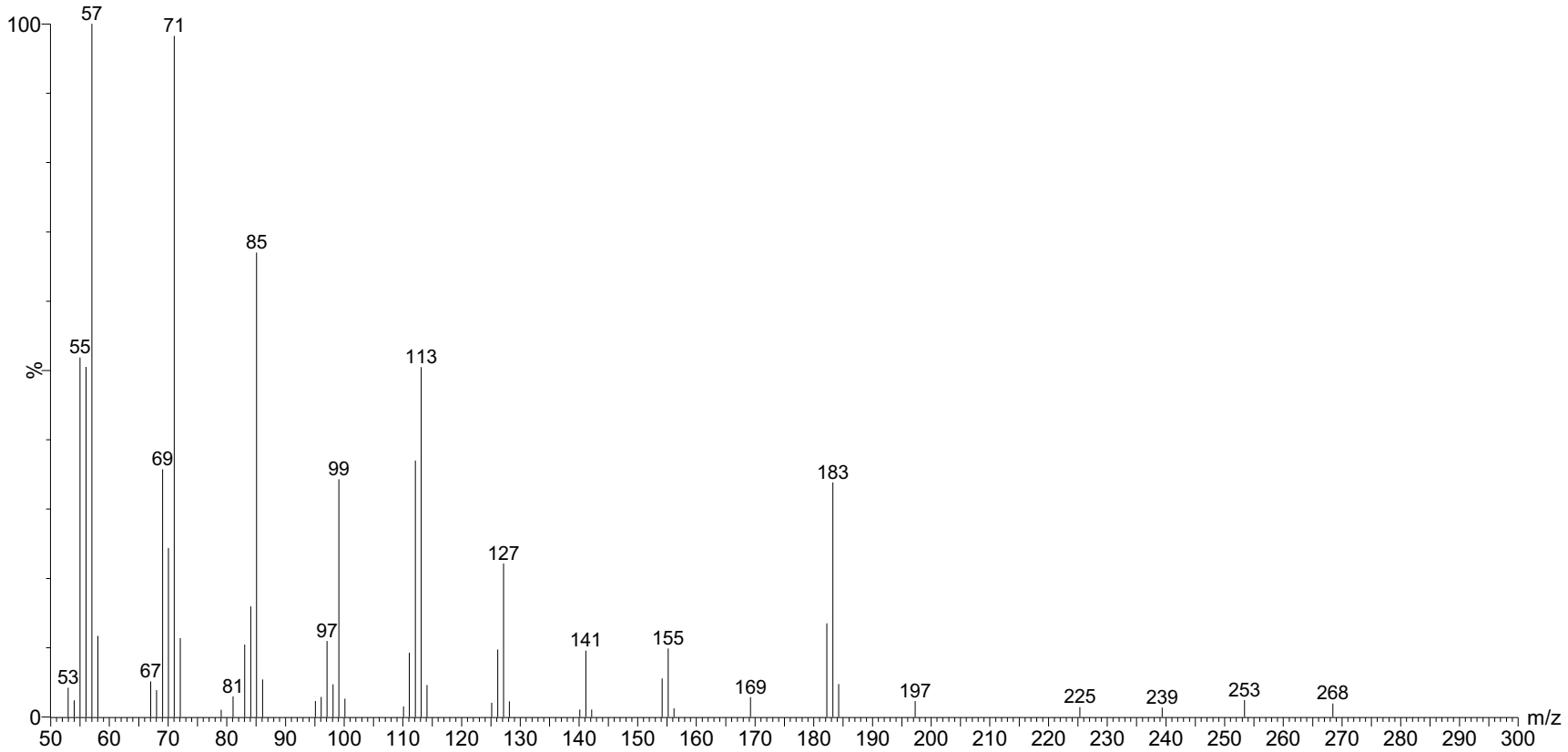
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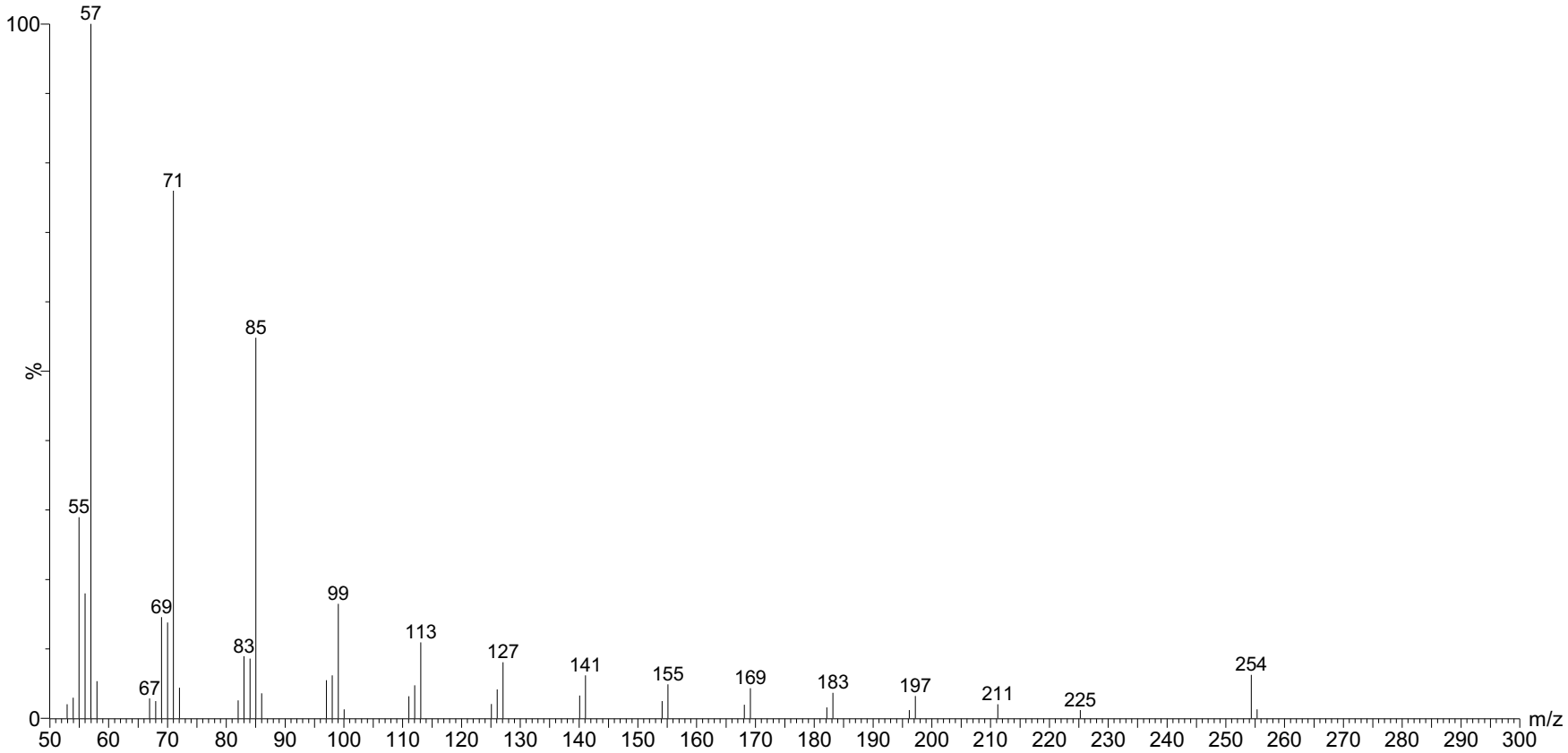
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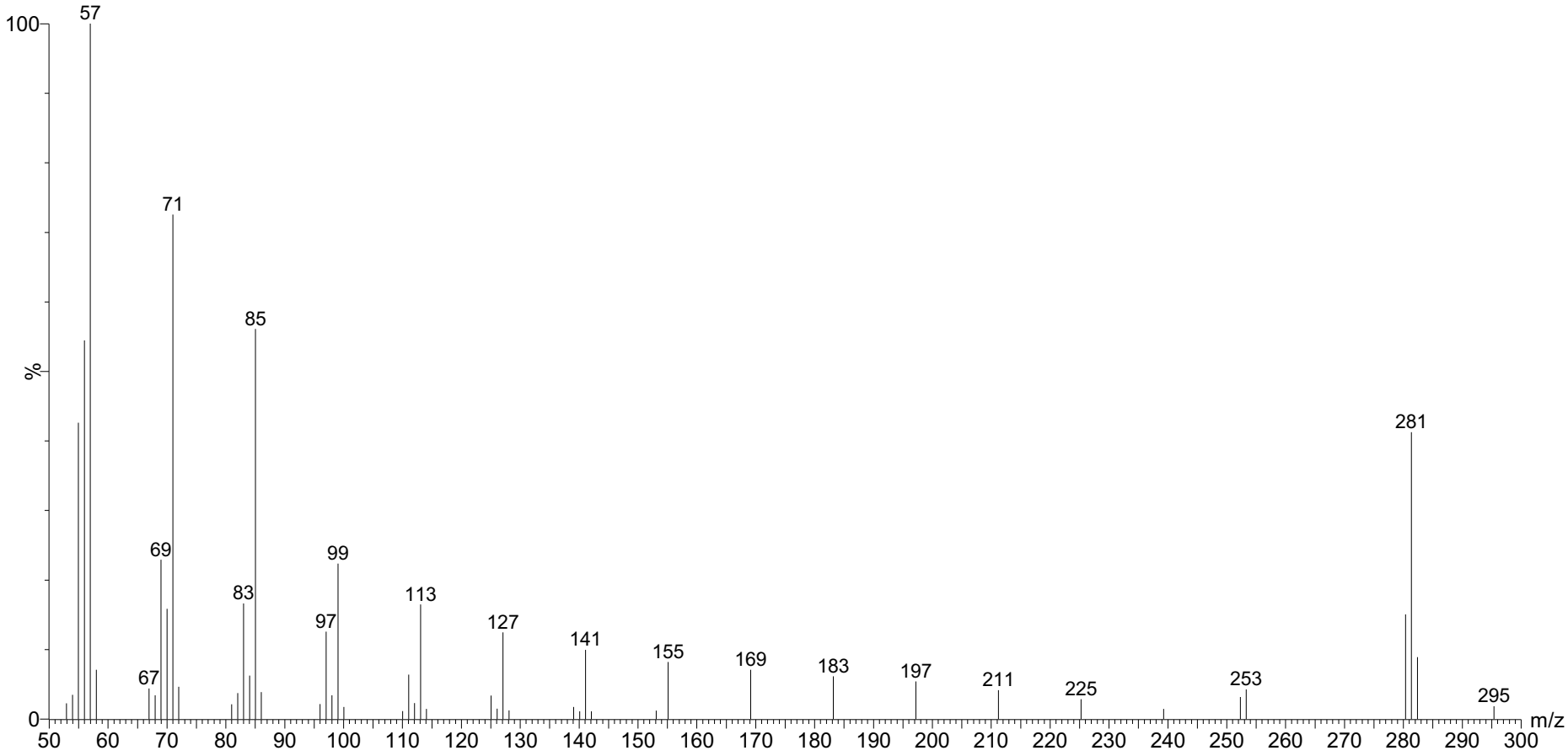
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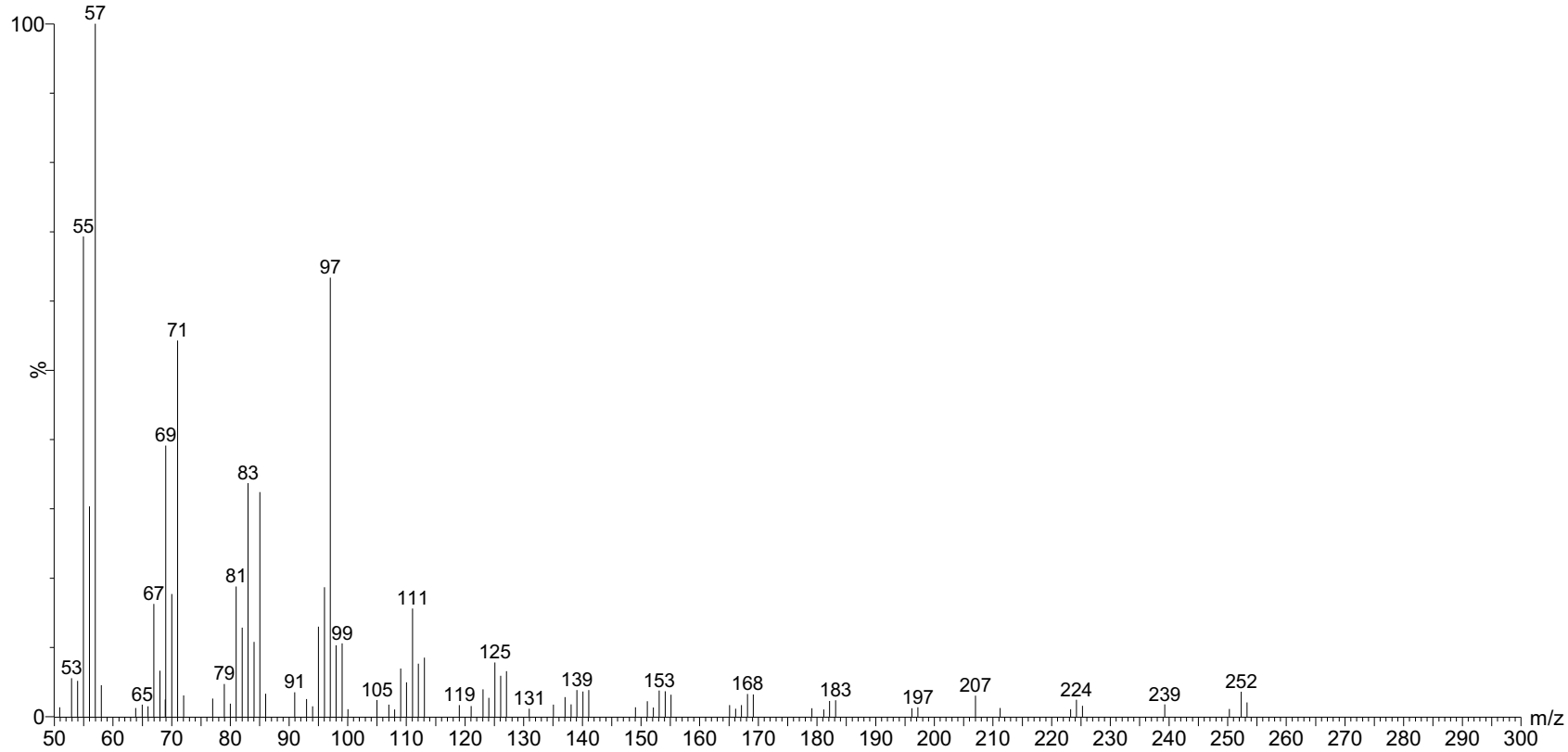
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m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300

Standard Interpretation Procedure

- Verify masses (calibration); determine elemental compositions & rings+unsaturations
- Mark odd electron ions and verify molecular ion
- Study appearance of spectrum, molecular stability, labile bonds
- Identify low mass ion series
- Identify neutral fragments, logical mass losses
- Postulate structures of low mass ions
- Postulate identity; test against ref. spectrum or library; rationalize fragmentation pattern

HPLC-ESI-MS data for Intact Polar Lipids

Water Column Biomass or Sediment:
Extract lipids (DCM: MeOH)

TLE

component separation by HPLC

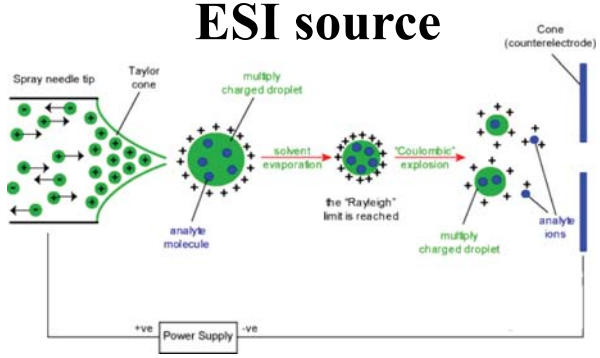
Density Map

Mass

Coeluting peaks make density map preferable

Retention time

ESI source



Mass spectrometer

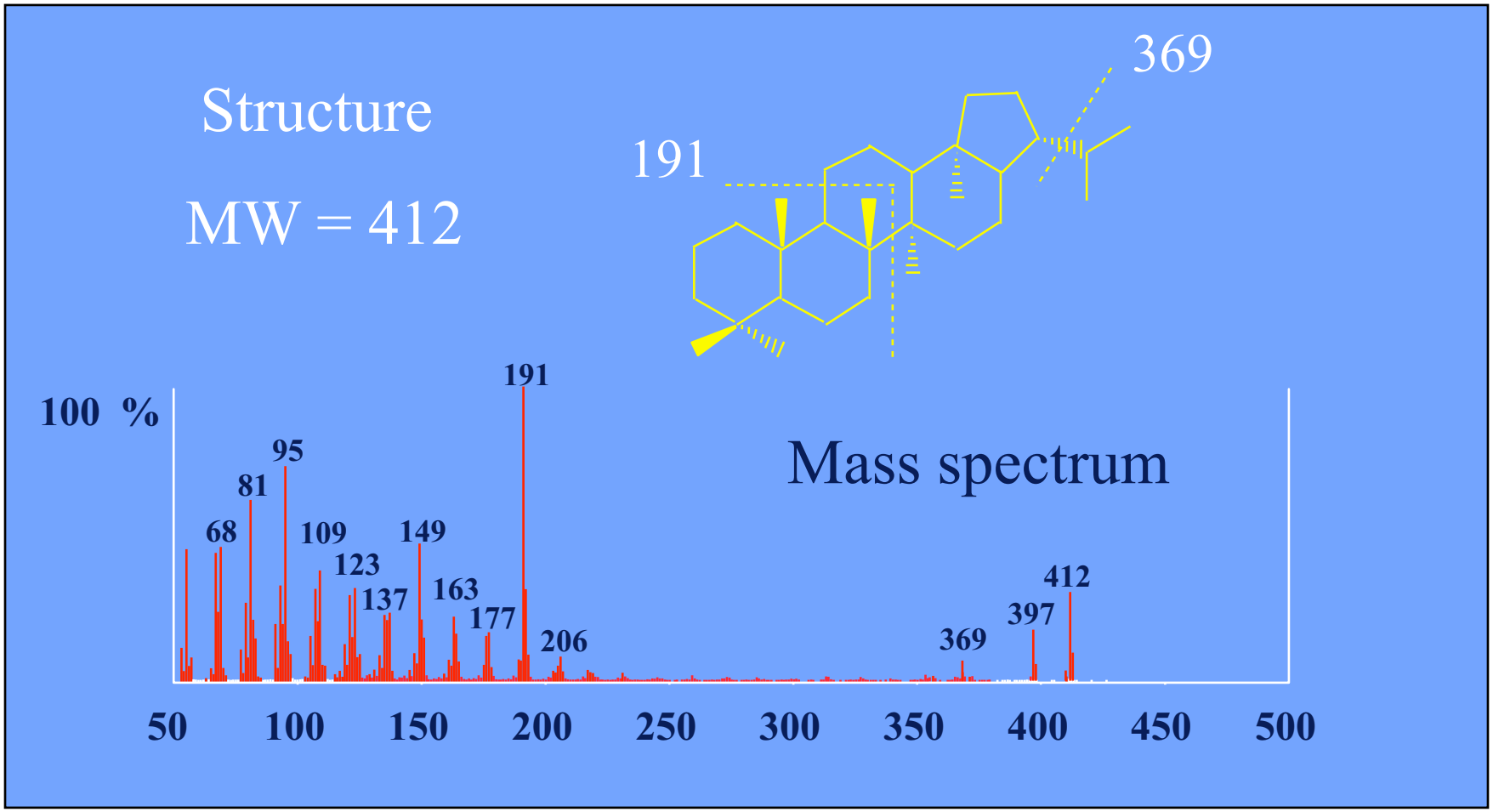
Detection of microbial biomass by intact polar membrane lipid analysis in the water column and surface sediments of the Black Sea

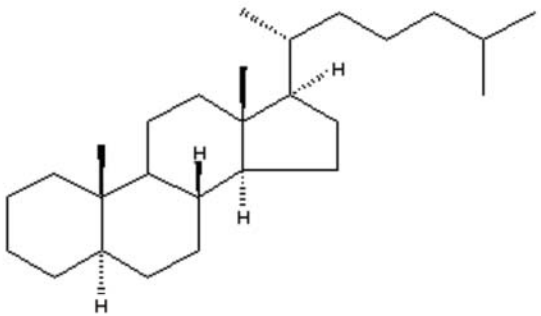
Florence Schubotz1, Stuart G. Wakeham, Julius S. Lipp, Helen F. Fredricks, Kai-Uwe Hinrichs

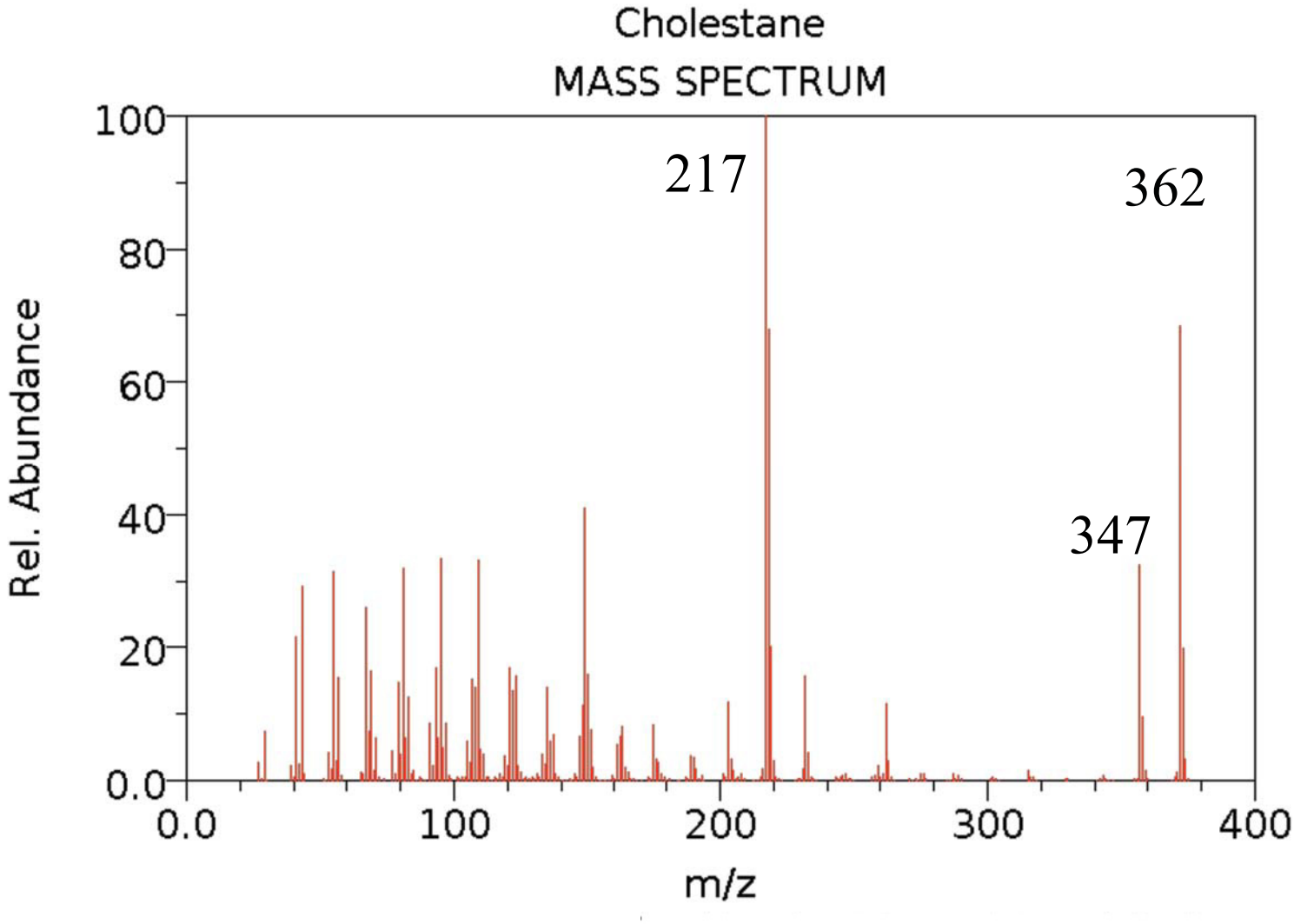
Environmental Microbiology11, 2720–2734, October 2009

GC-MS of fossil hydrocarbons

αβ-Hopane a fossil hydrocarbon

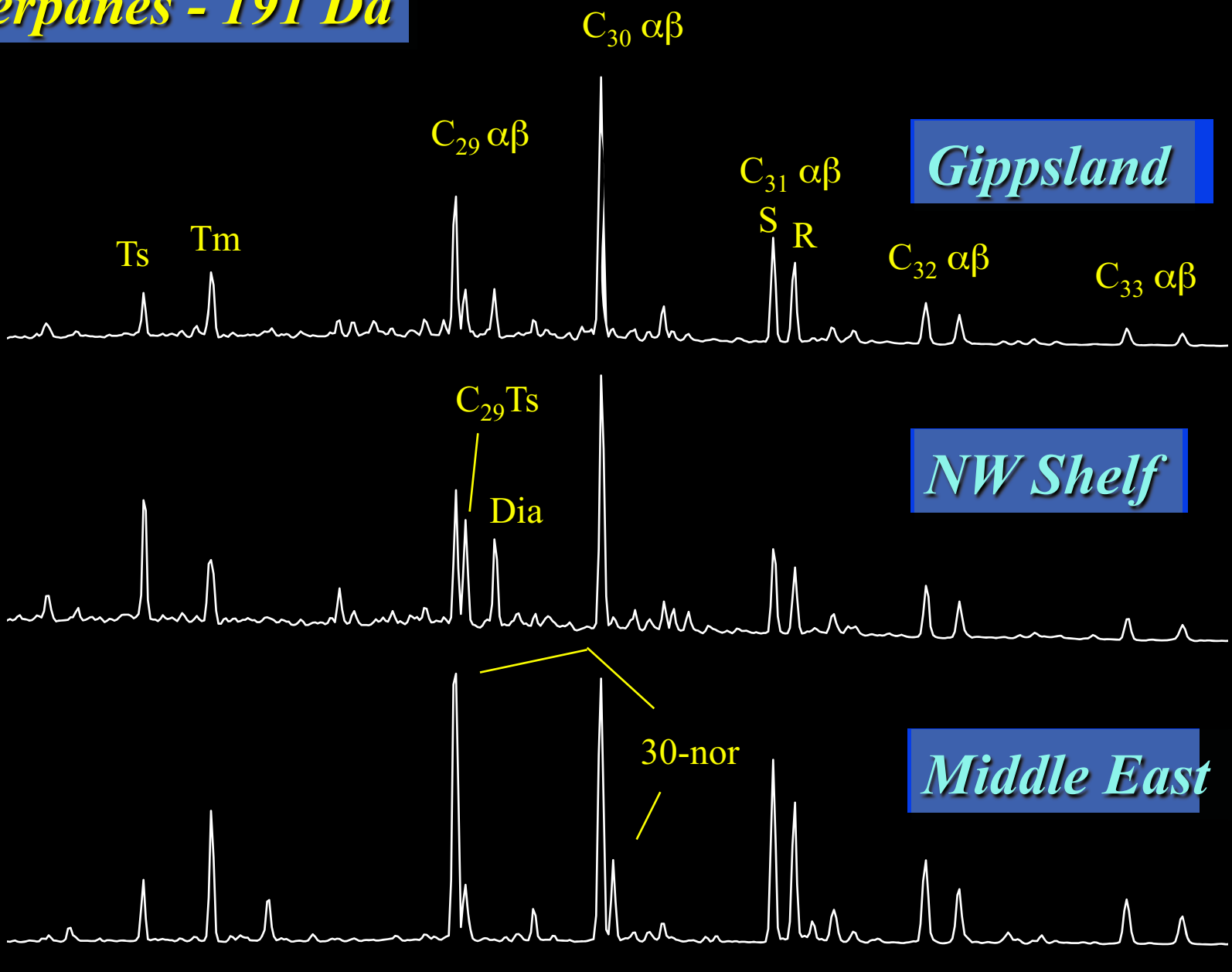






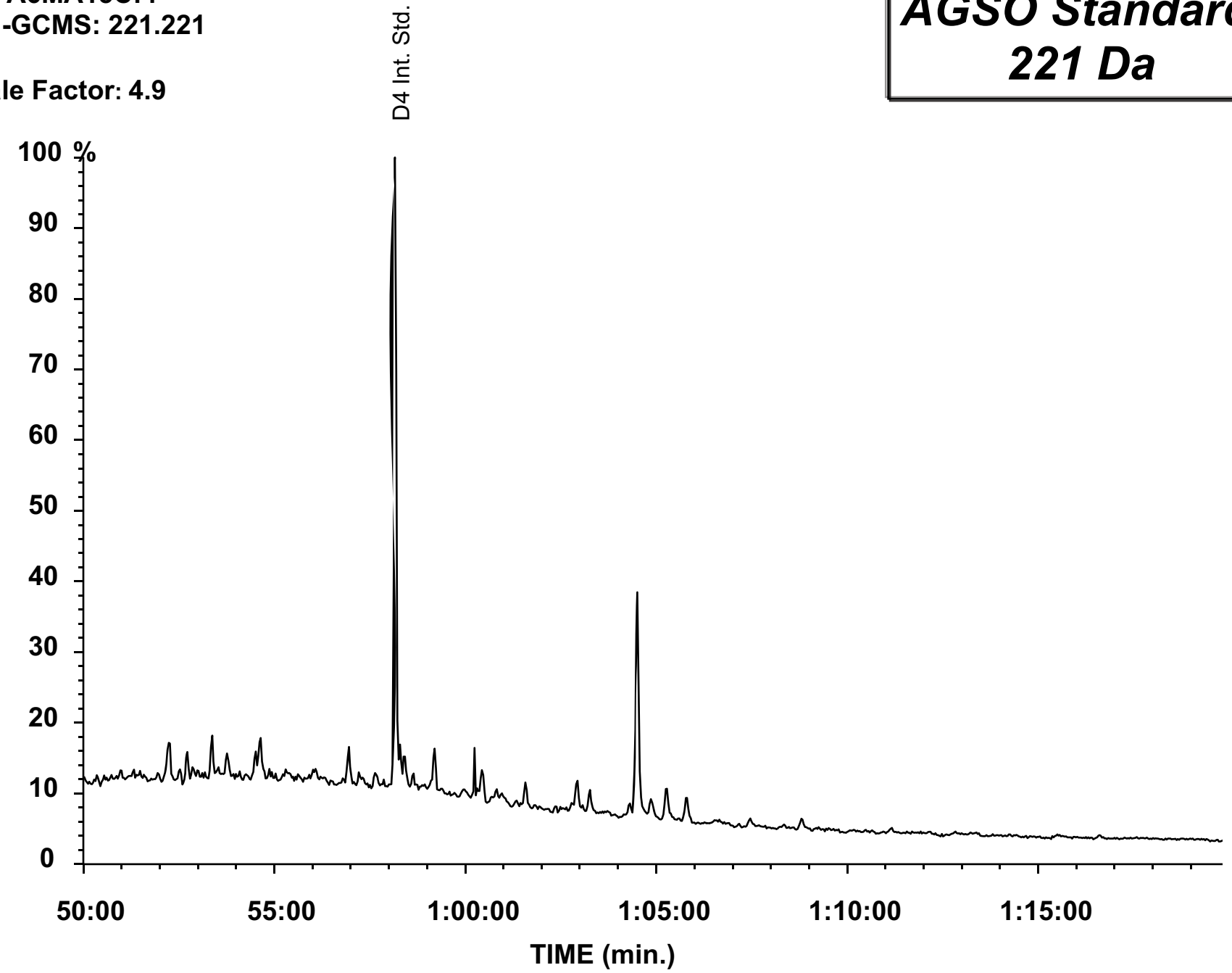
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Triterpanes - 191 Da



File A6MA15C:4
SIM-GCMS: 221.221
Da
Scale Factor: 4.9

AGSO Standard 221 Da



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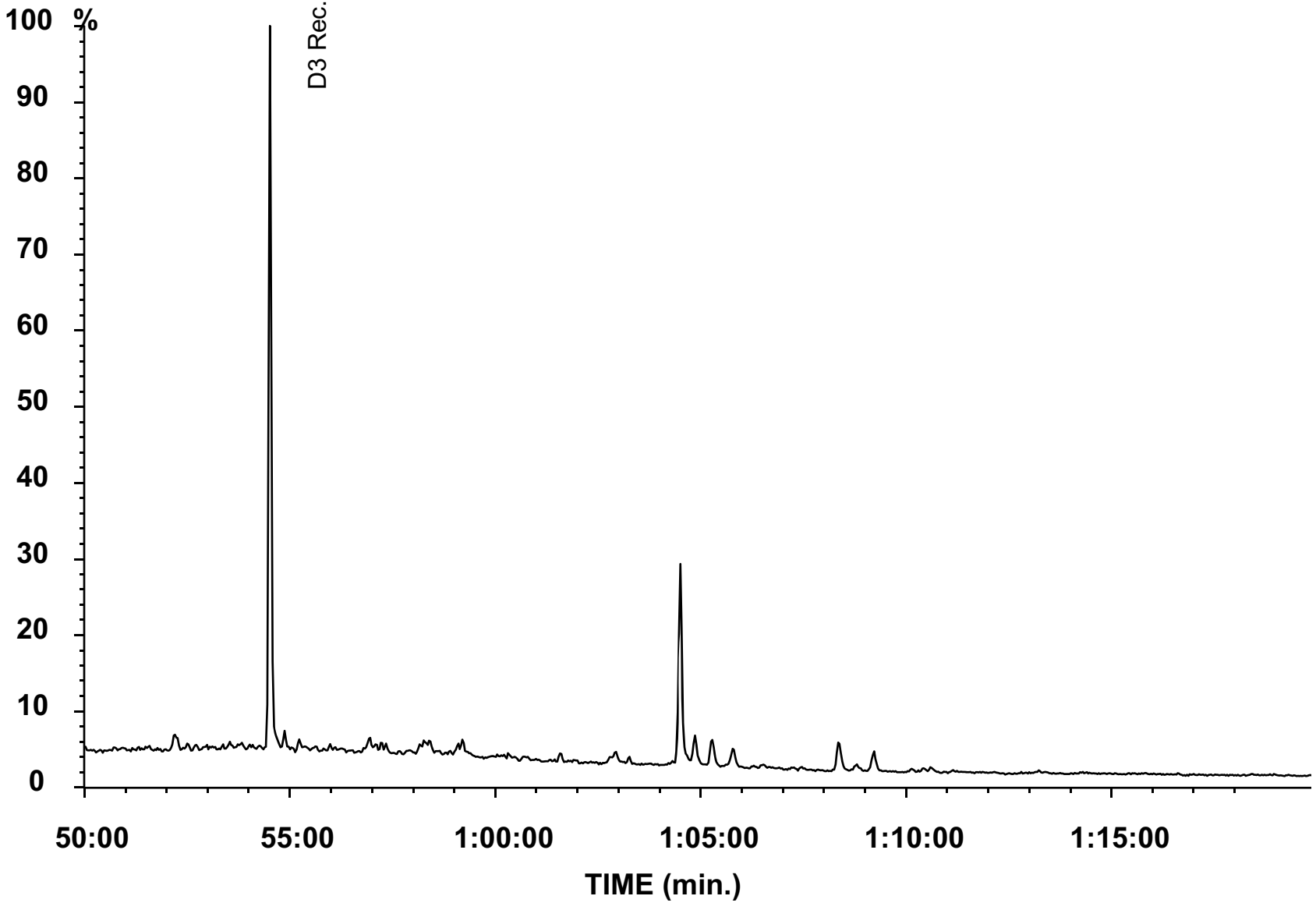
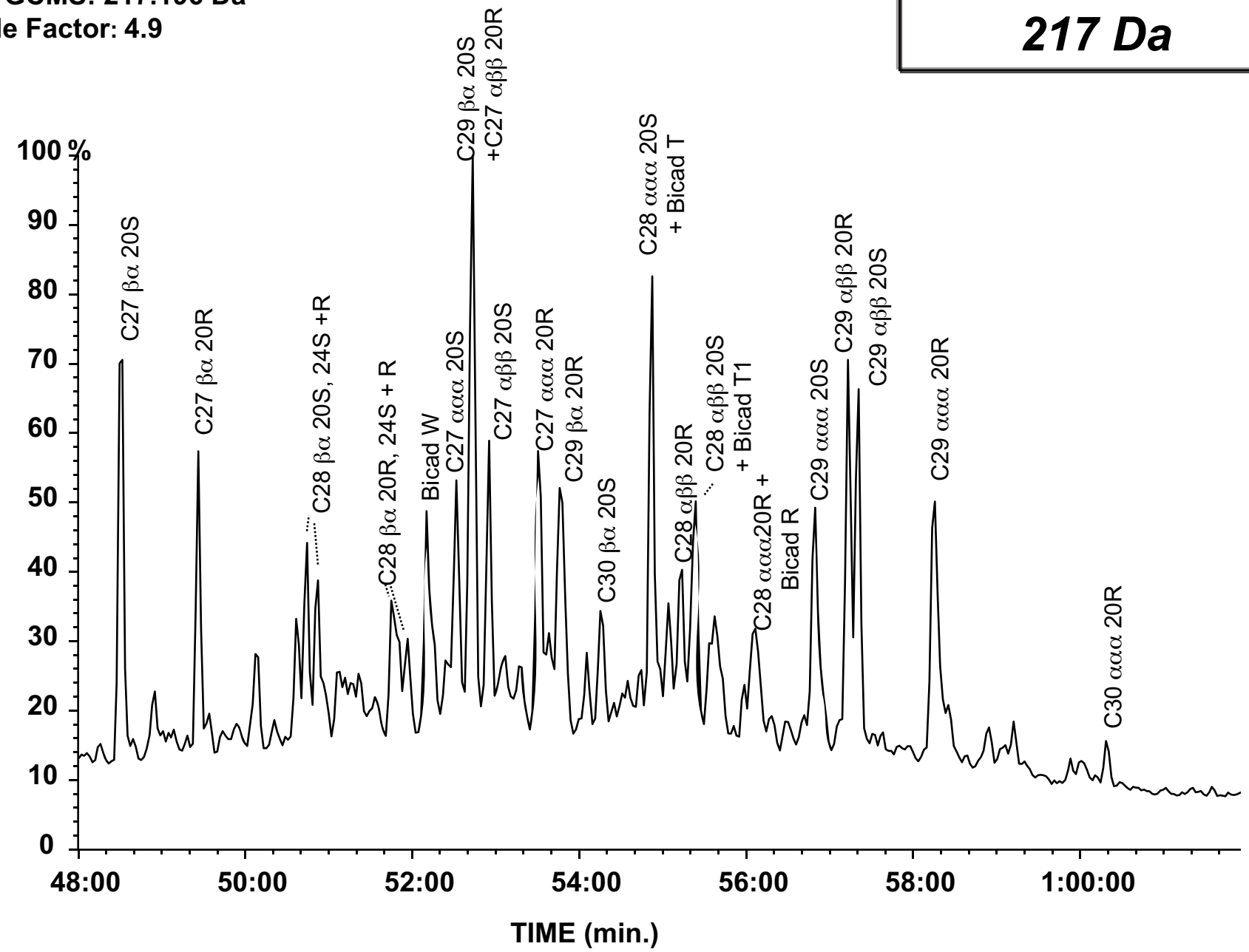
SIM-GCMS: 234.232 Da
Scale Factor: 5.4

AGSO Standard 234 Da

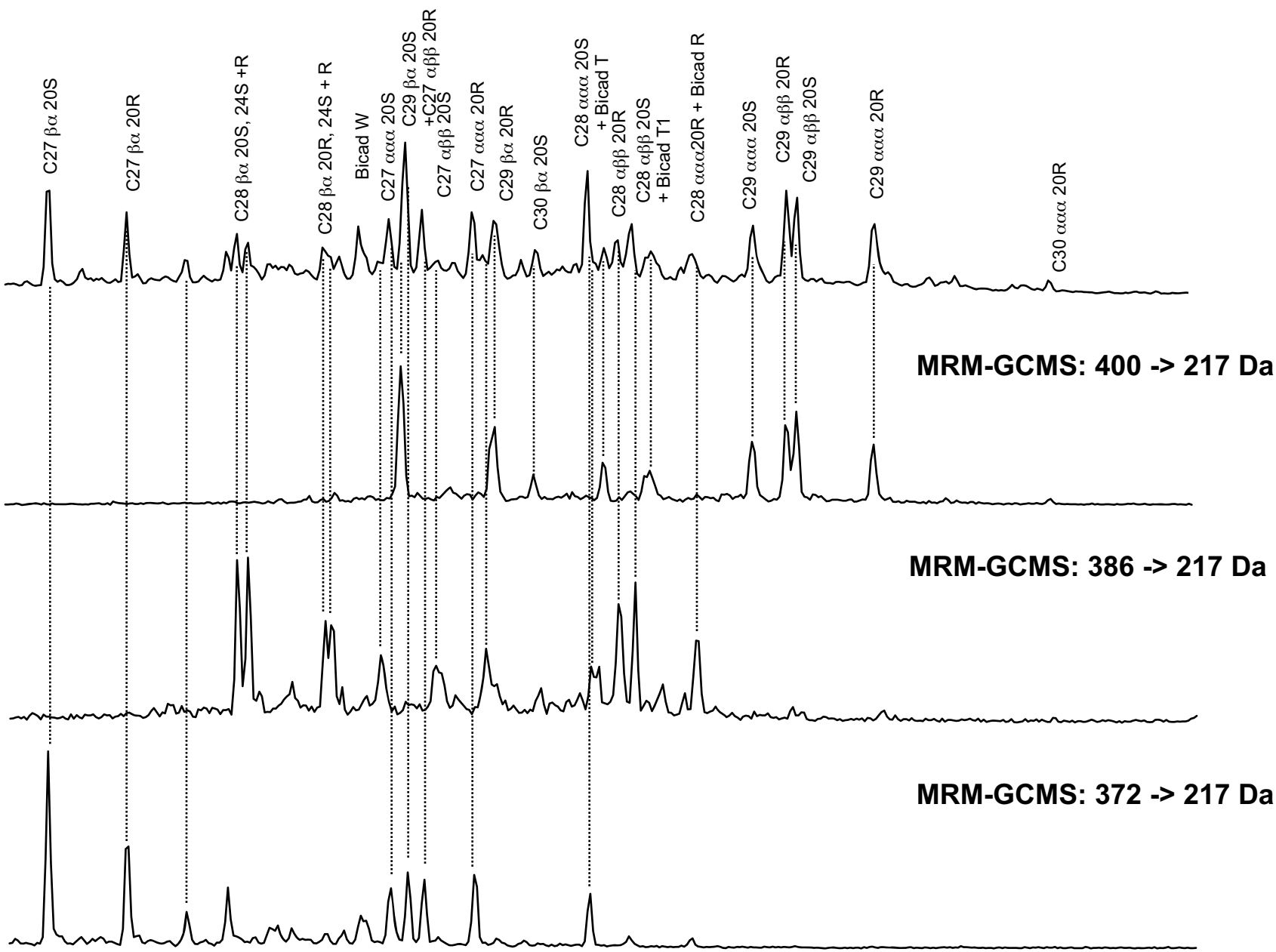


File A6MA15C:4
SIM-GCMS: 217.196 Da
Scale Factor: 4.9

AGSO Standard 217 Da



AGSO Standard: steranes SIM vs. MRM



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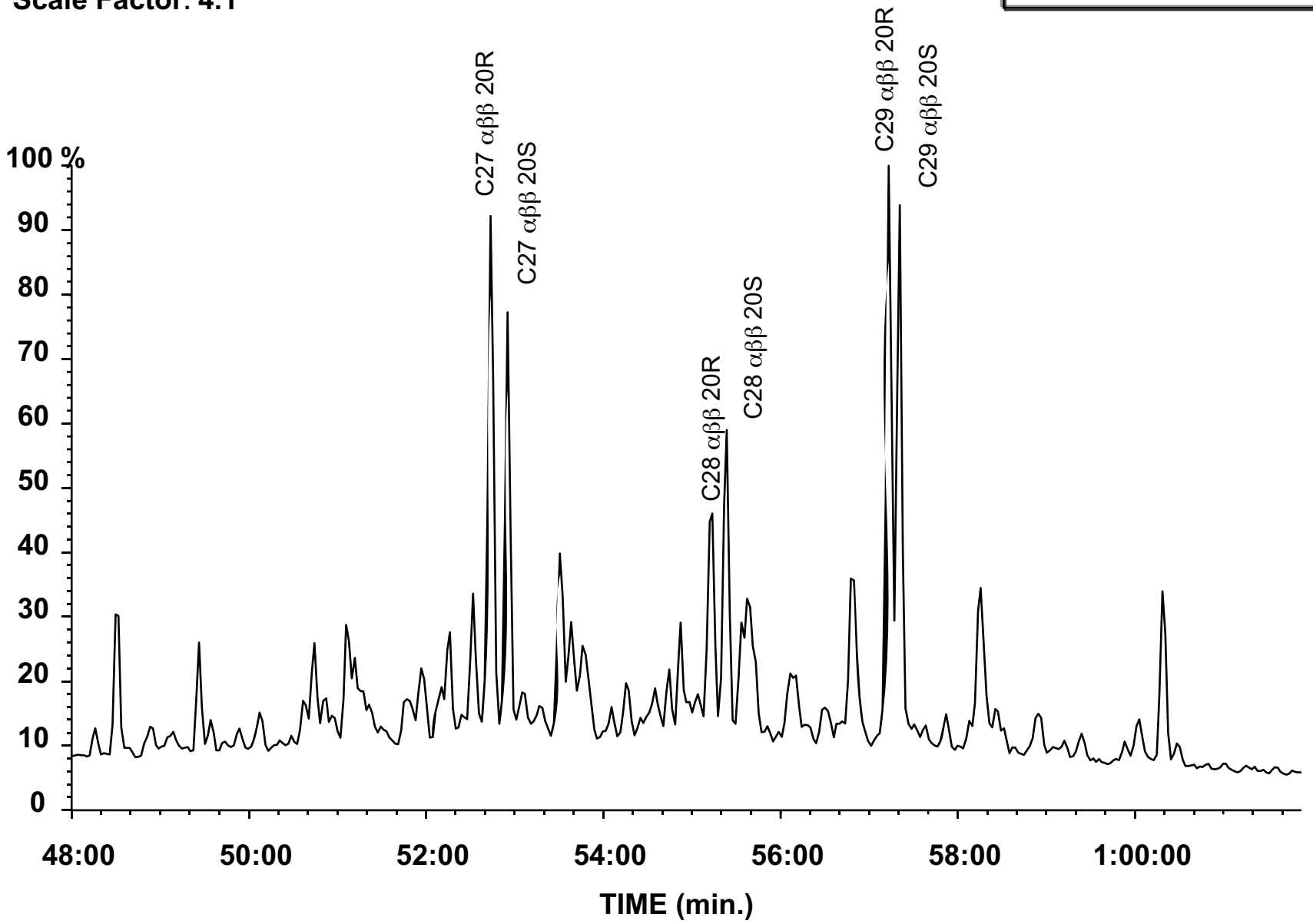
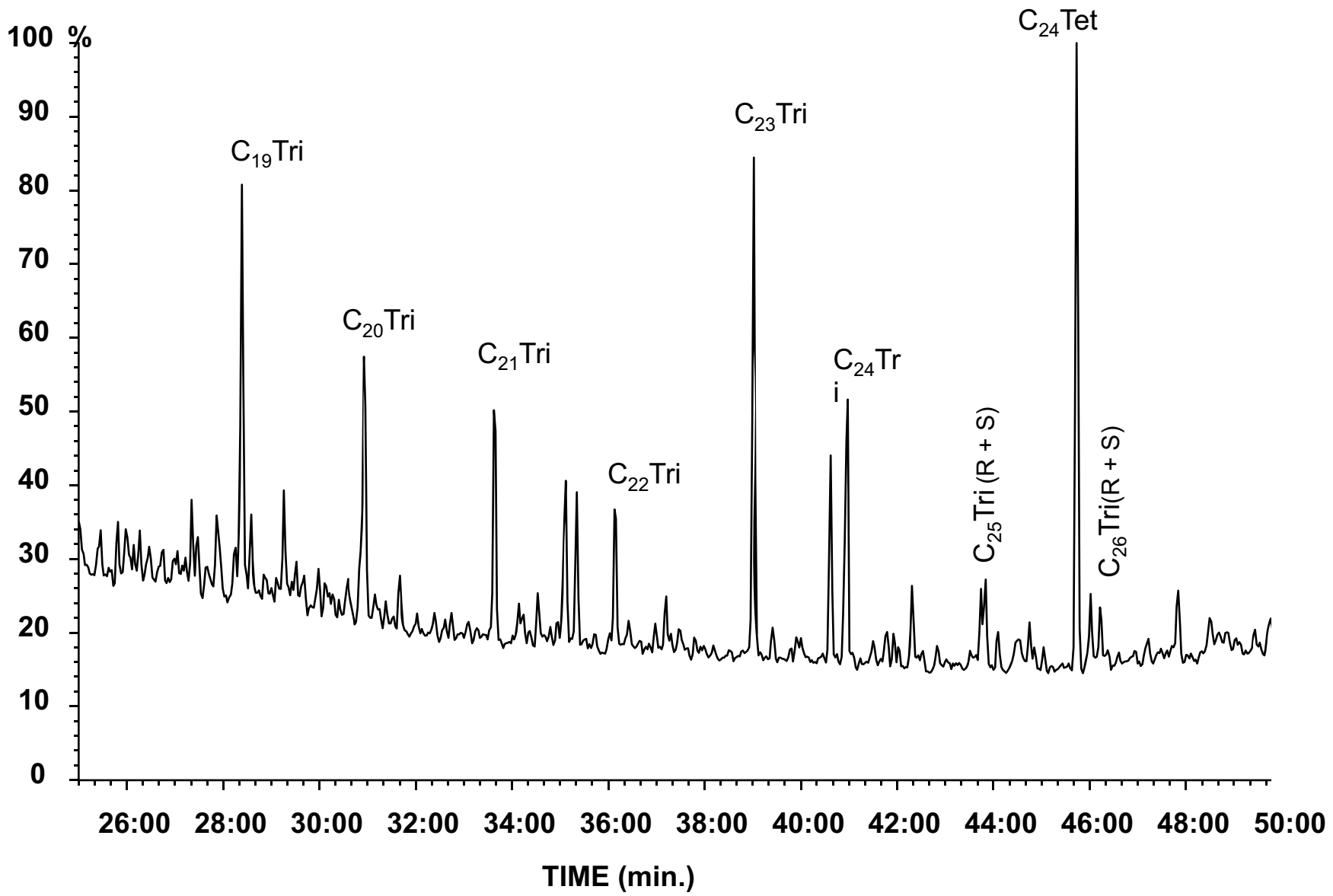
SIM-GCMS: 218.203
Da
Scale Factor: 4.1

AGSO Standard 218 Da



File A6MA15C:4
SIM-GCMS: 191.179
Da
Scale Factor: 6.4

AGSO Standard 191 Da



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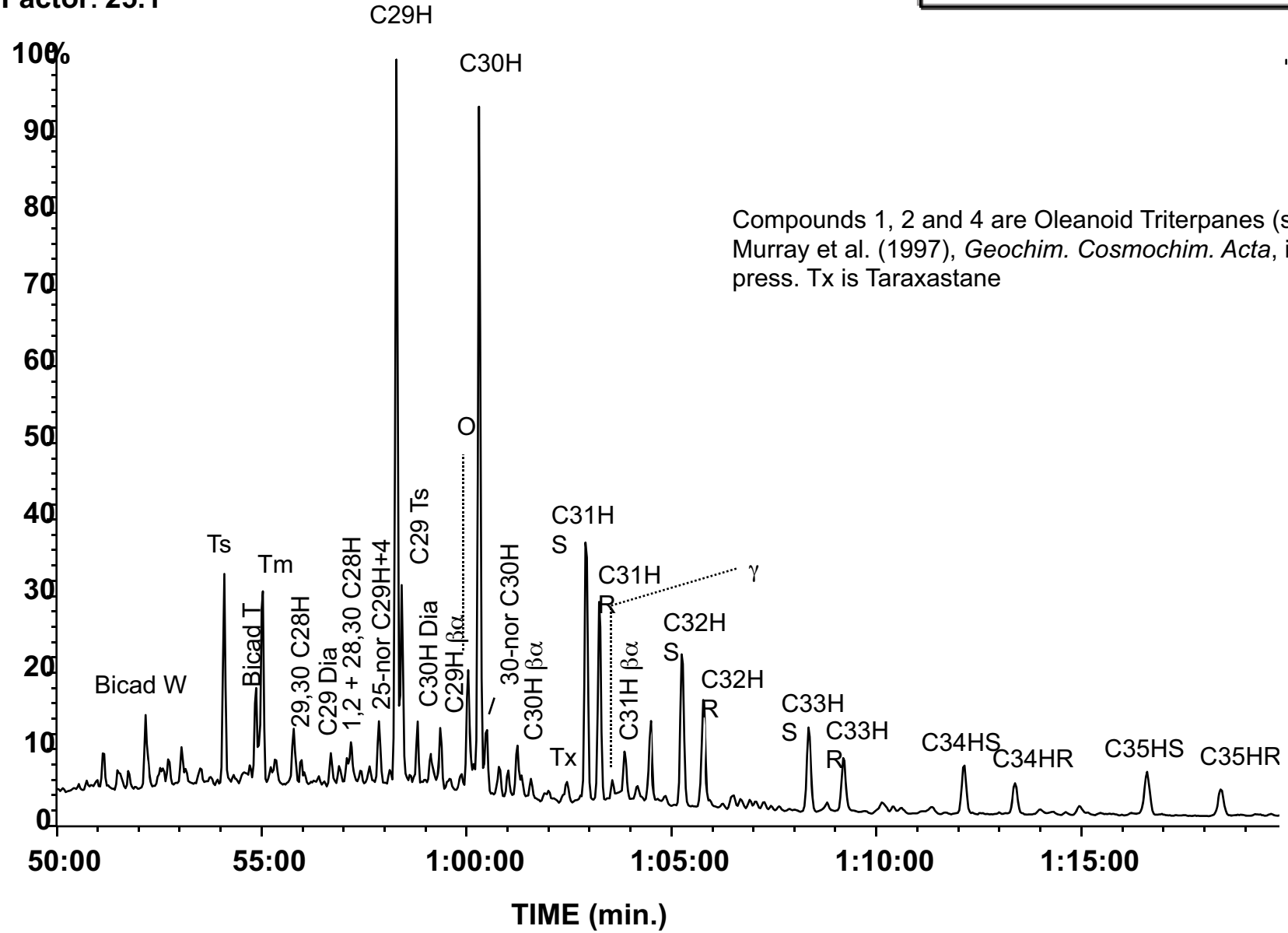
SIM-GCMS: 191.179 Da
Scale Factor: 25.1

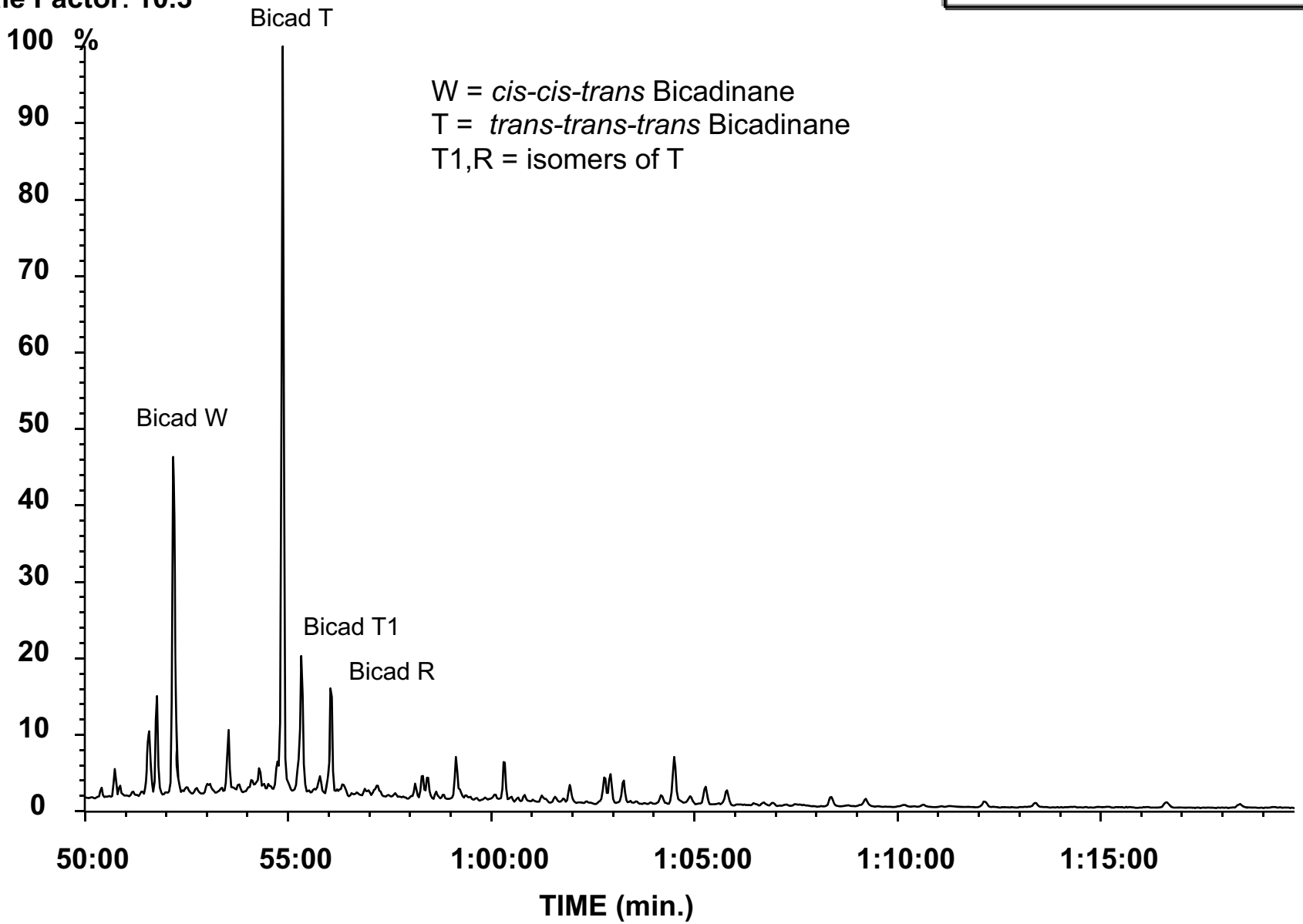
AGSO Standard 191 Da



Compounds 1, 2 and 4 are Oleanoid Triterpanes (see Murray et al. (1997), *Geochim. Cosmochim. Acta*, in press. Tx is Taraxastane

File A6MA15C:4
SIM-GCMS: 369.352 Da
Scale Factor: 10.3

AGSO Standard 369 Da

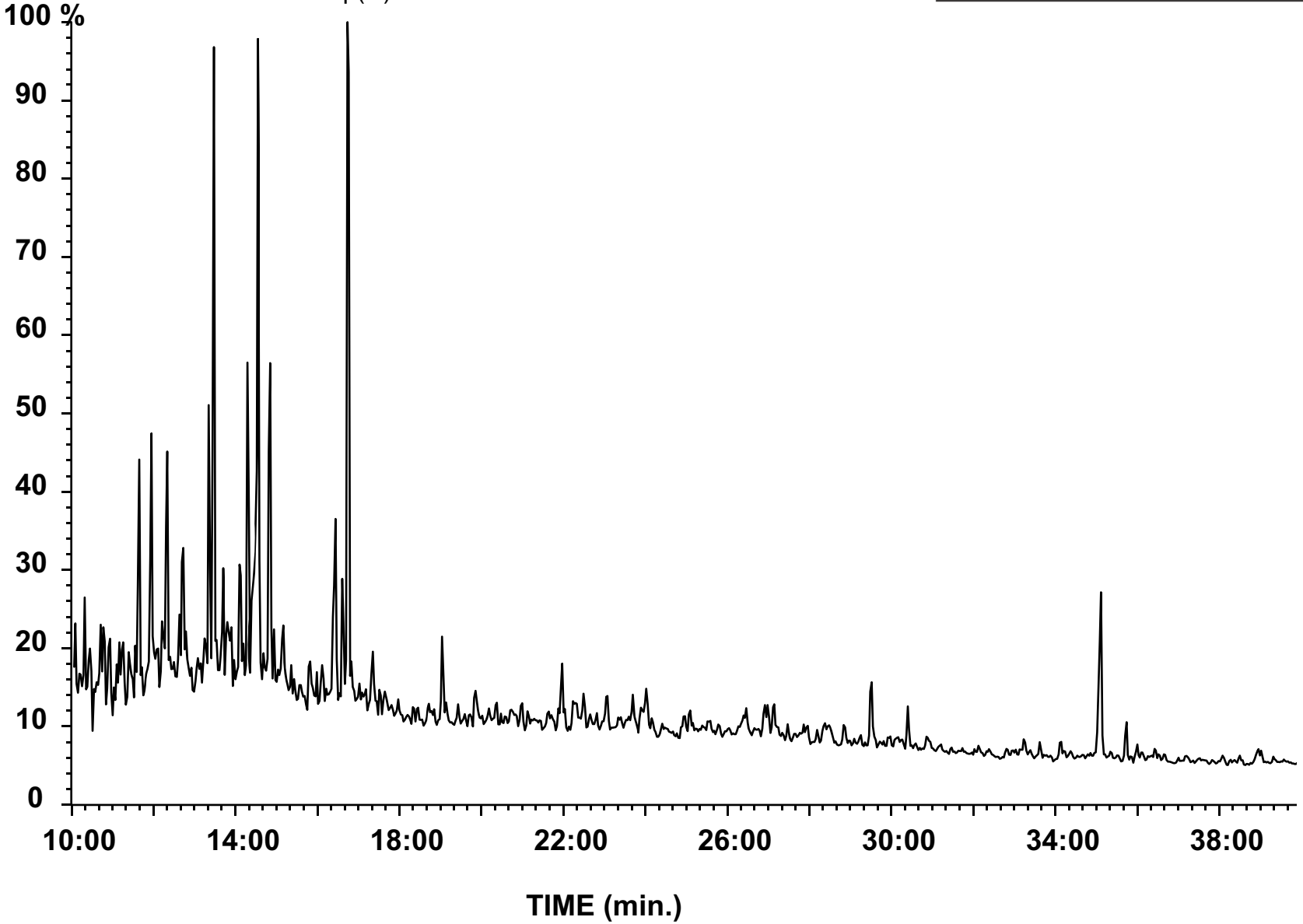


W = *cis-cis-trans* Bicadinane
T = *trans-trans-trans* Bicadinane
T1,R = isomers of T

File A6MA15C:4
SIM-GCMS: 123.117 Da
Scale Factor: 100

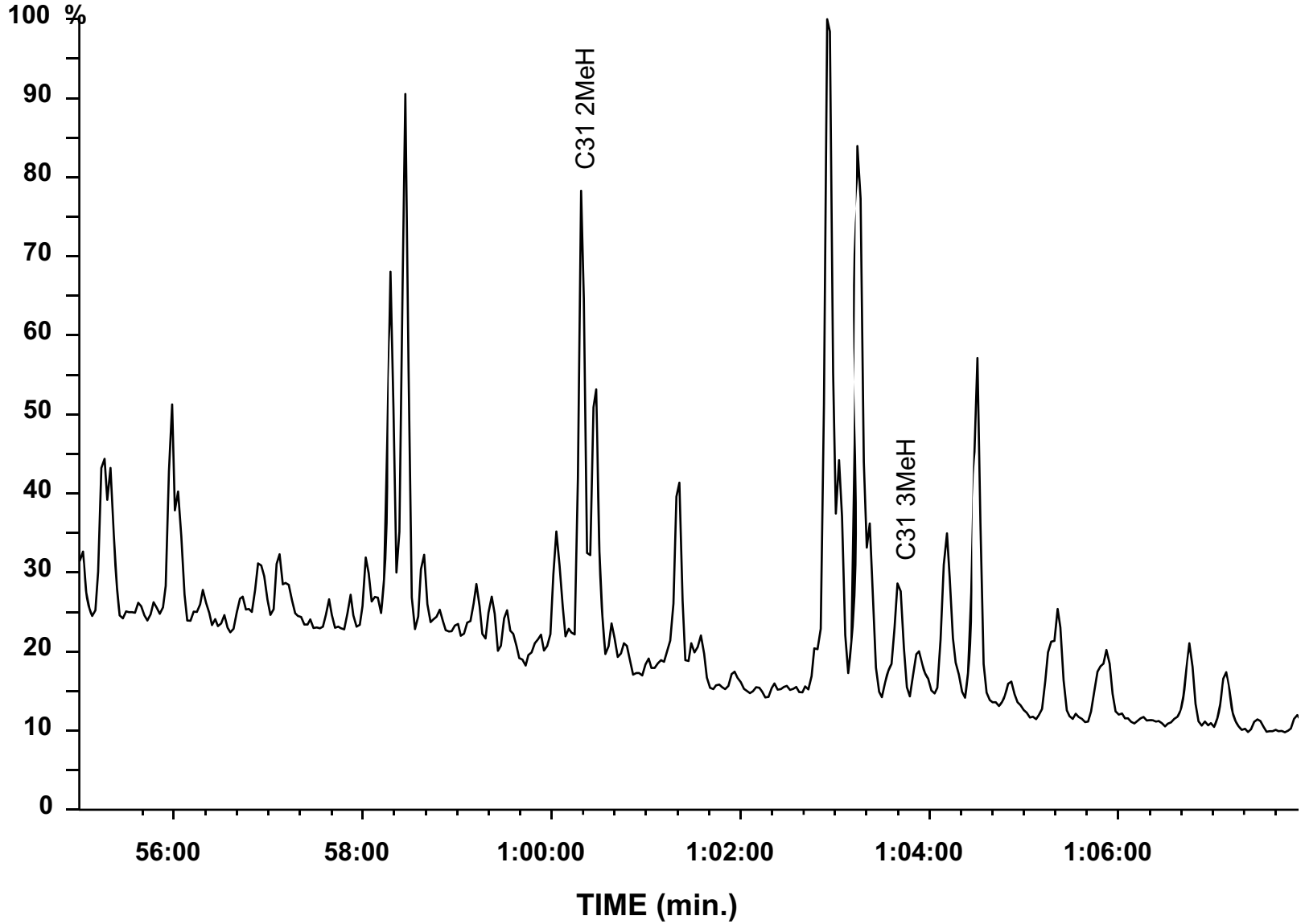
8 β(H)-Homodrimane

AGSO Standard 123 Da



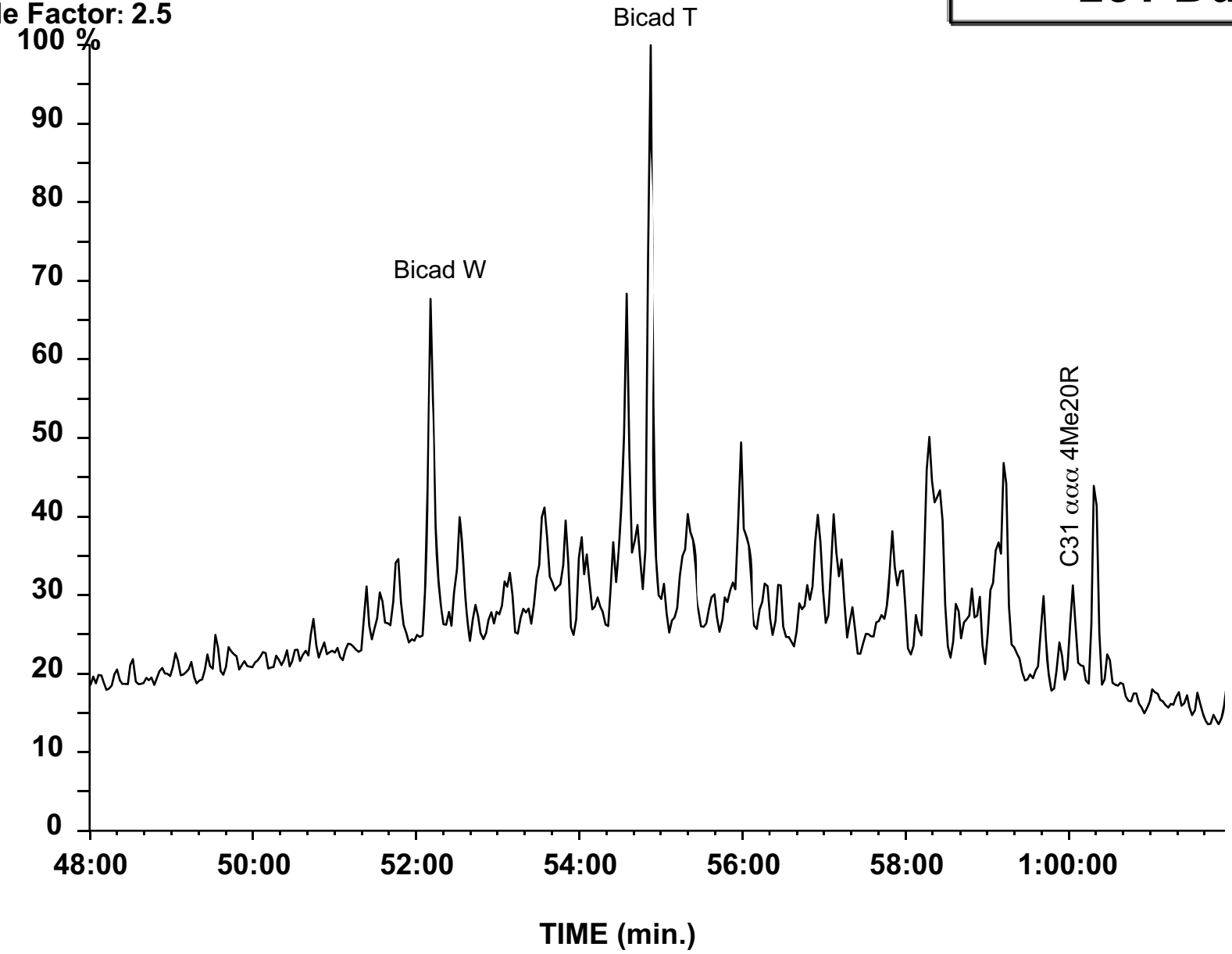
File A6MA15C:4
SIM-GCMS: 205.194 Da
Scale Factor: 10.3

AGSO Standard 205 Da



File A6MA15C:4
SIM-GCMS: 231.212 Da
Scale Factor: 2.5

AGSO Standard 231 Da



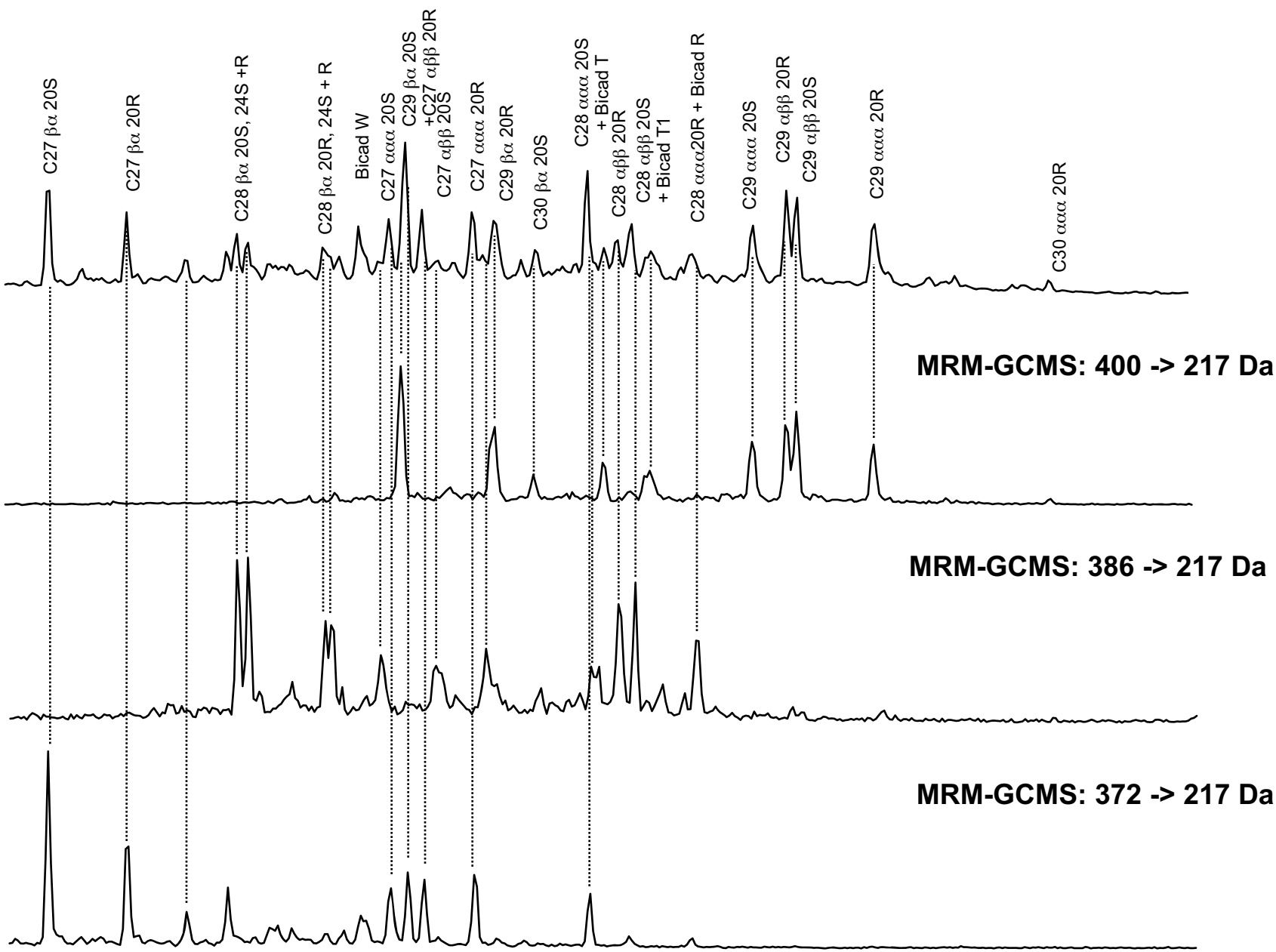
GC-MS-MS

True GC-MS-MS requires an instrument with at least two, independently controllable mass selection regions.

Various configurations possible, e.g. Triple Sector Quadruple,"Hybrid" magnet/ quadrupole etc. e.g. Autospec-Q, Finnagin MAT 95Q etc.

Very high sensitivity and selectivity very powerful for biomarker work.

AGSO Standard: steranes SIM vs. MRM



MRM-GC-MS

Metastable Reaction Monitoring GC-MS Monitoring of ion reactions (e.g. Molecular-Daughter) by selecting metastable ions in the first field free region.

Not "true" GCMSMS but allows selective recording of biomarker molecular weight groups (e.g. *only* C_{30} steranes *cf.* all steranes at once).

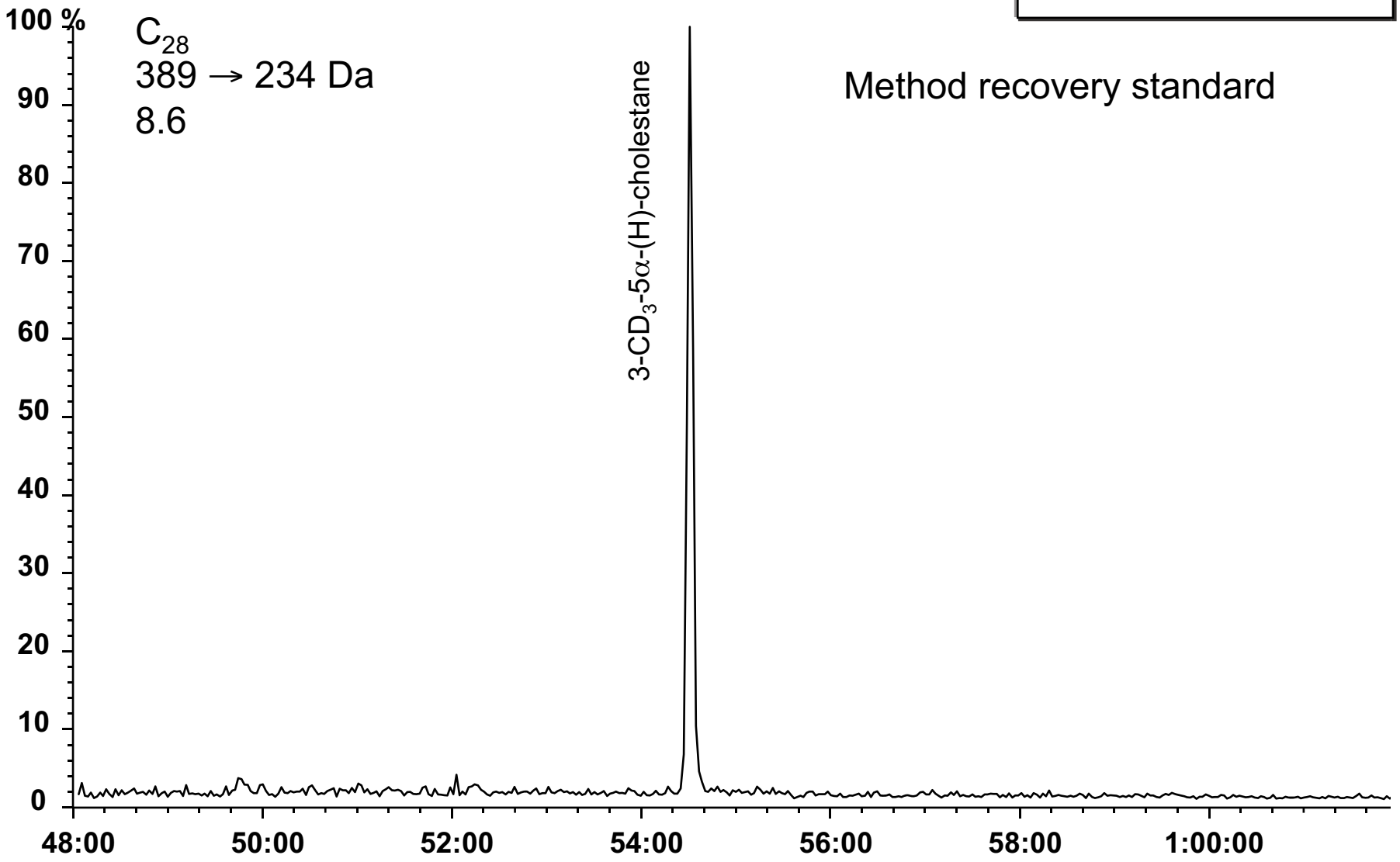
MRM-GC-MS (2)

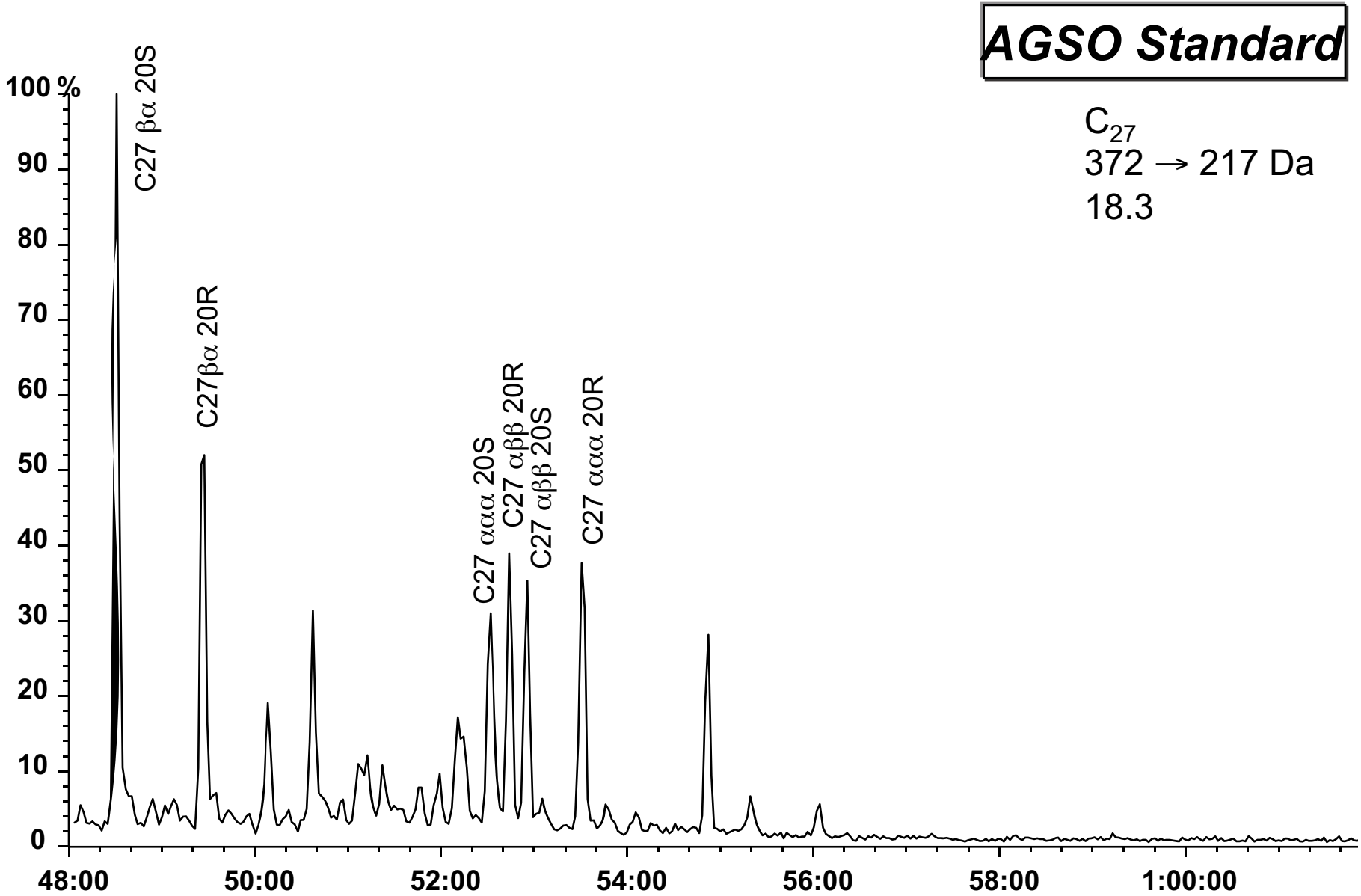
Metastable Reaction Monitoring GC-MS

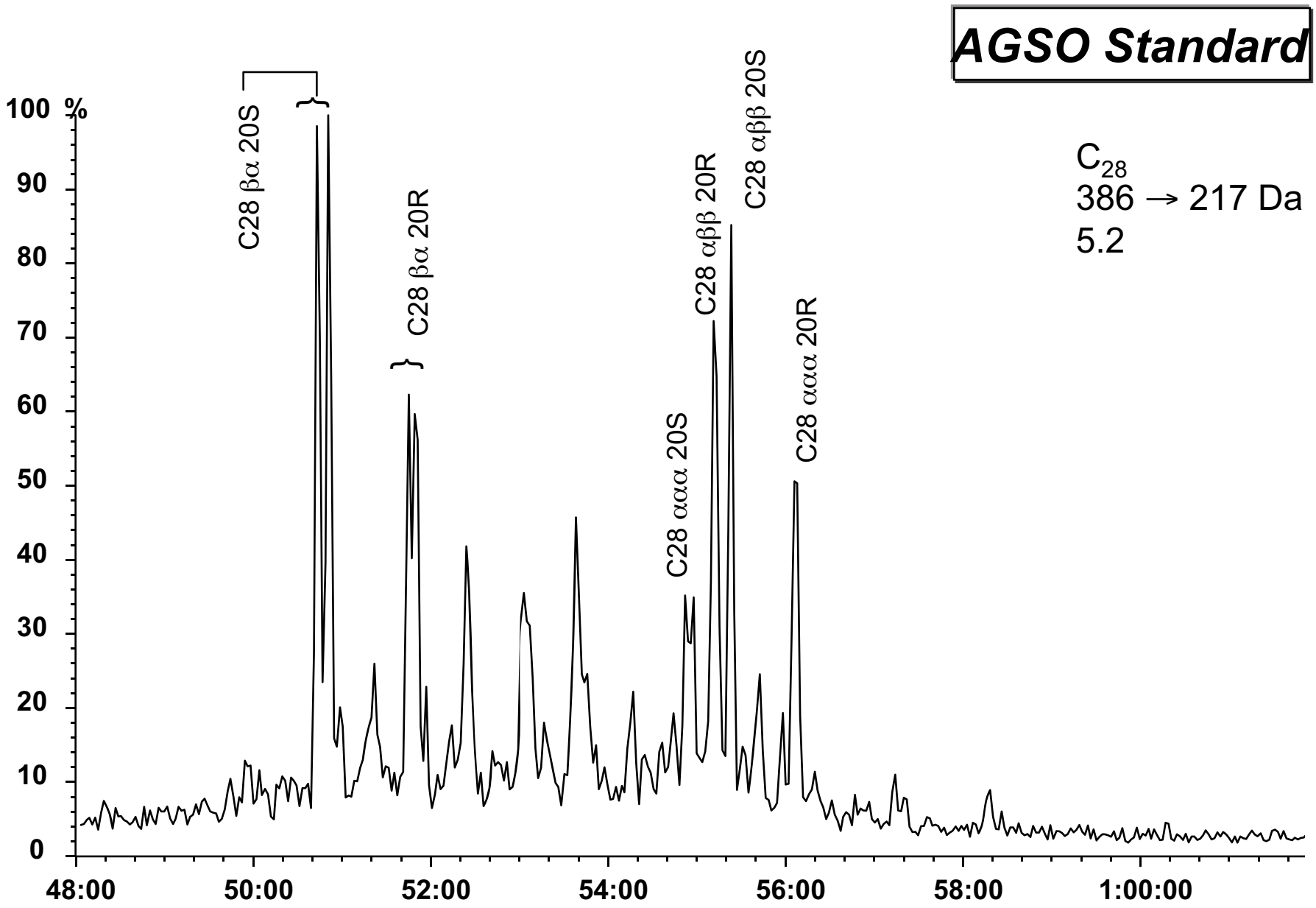
Higher selectivity and sensitivity.

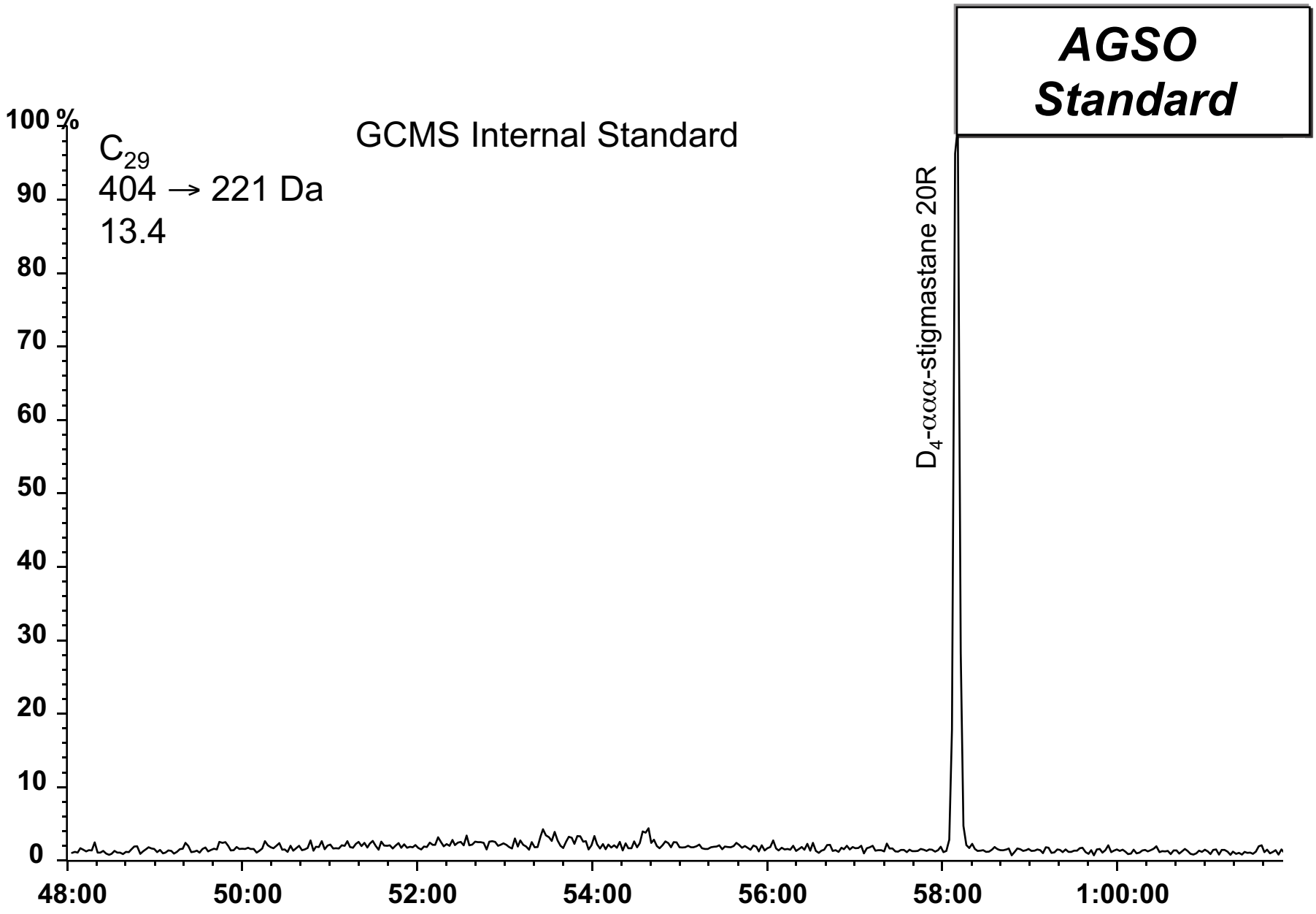
Can be done on double focussing, high resolution instruments such as VG 70E etc but parent ions can only be selected at low resolution - some interference or "cross talk" between traces.

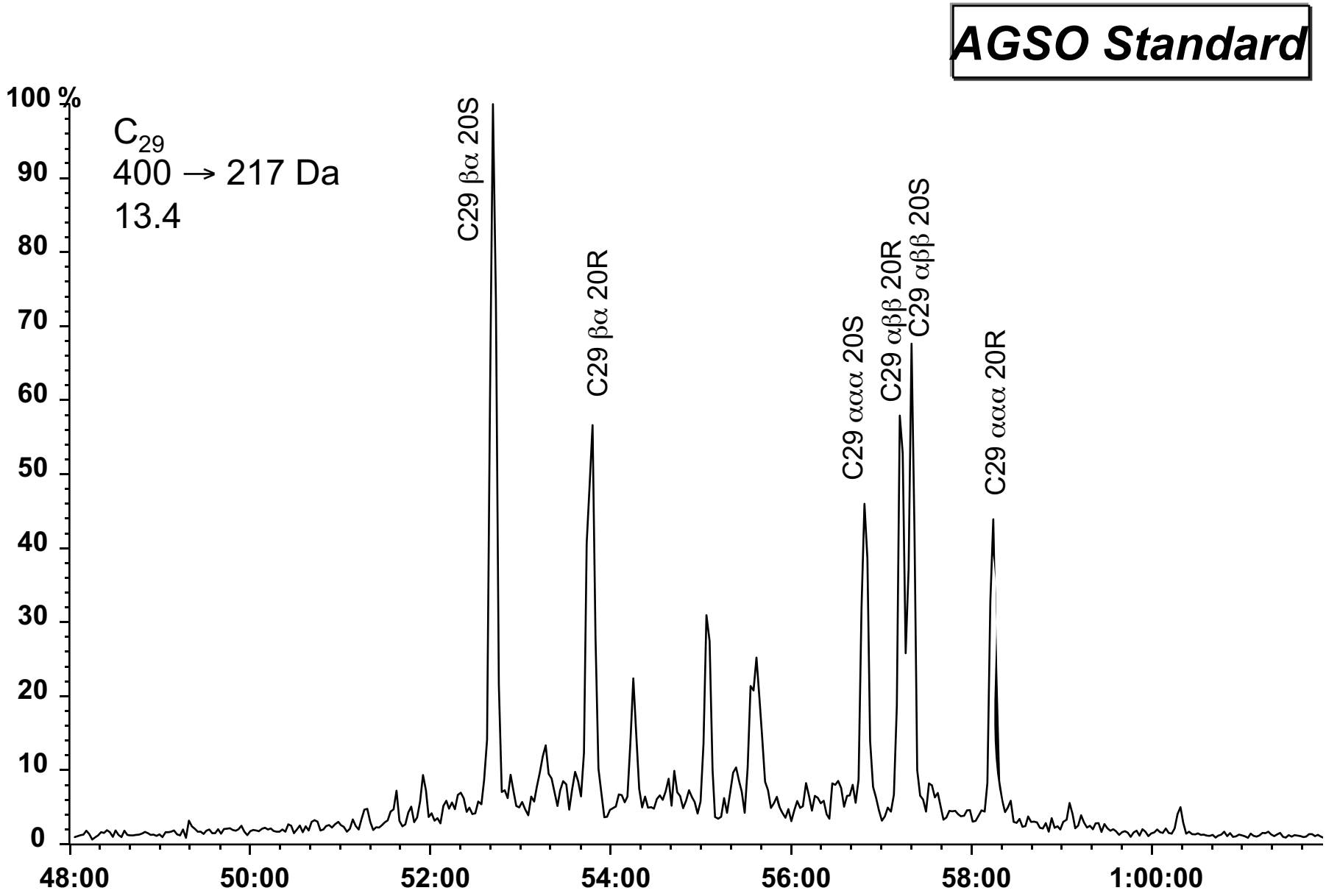
AGSO Standard

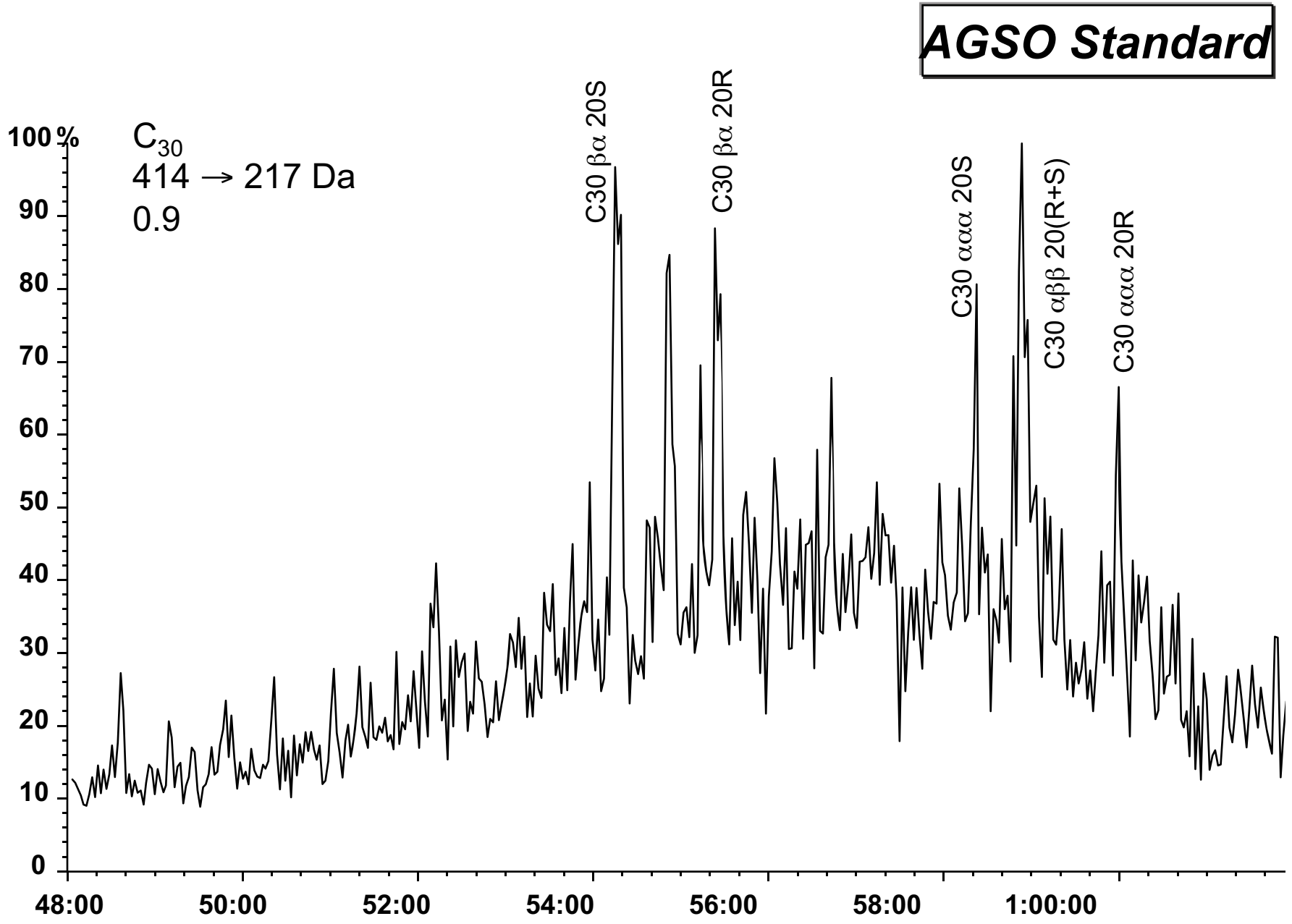



AGSO Standard
C27
372 → 217 Da
18.3
100 %
90
80
70
60
50
40
30
20
10
0
C27 βα 20S
C27βα 20R
C27 ααα 20S
C27 αββ 20R
C27 αββ 20S
C27 ααα 20R
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

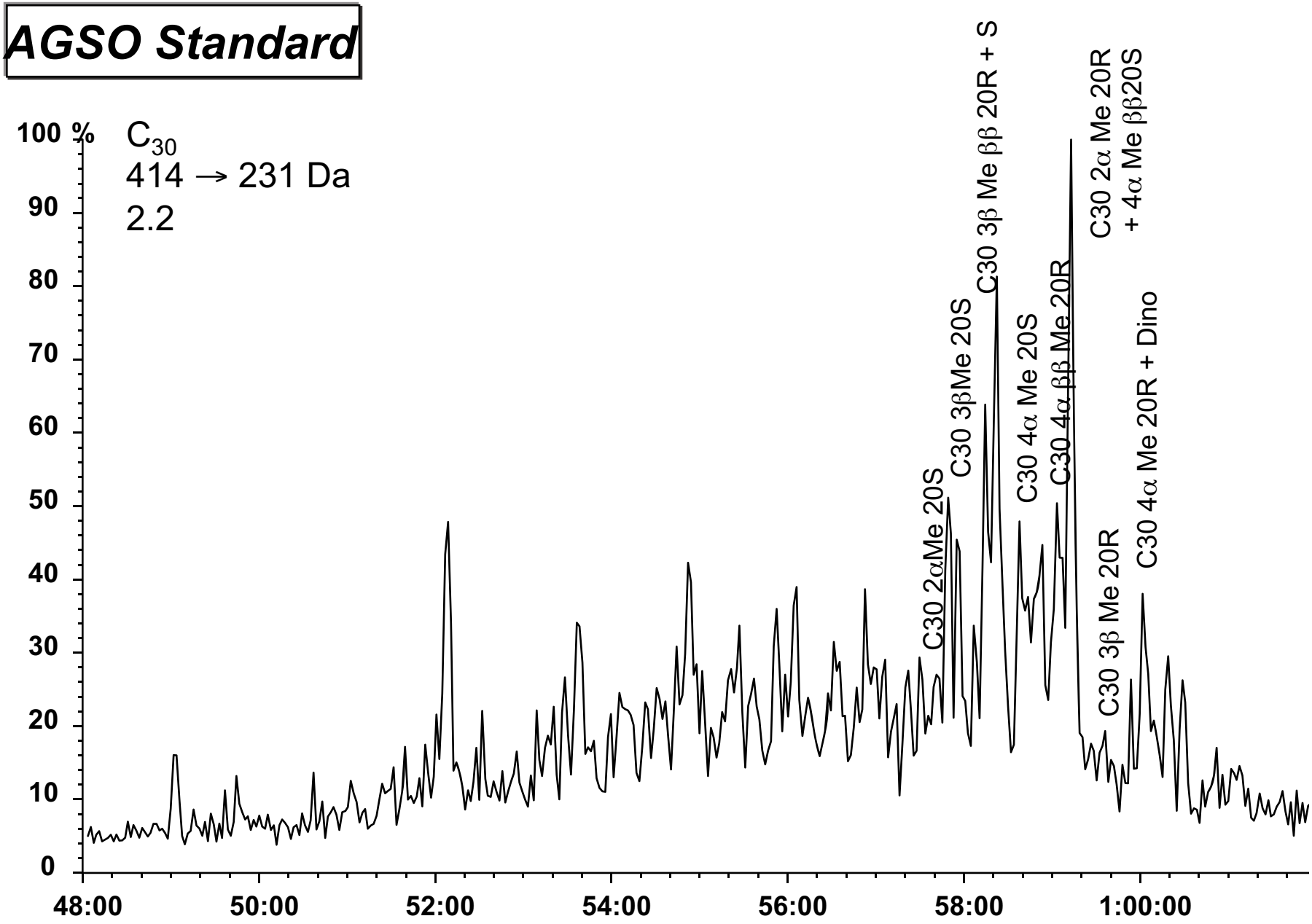

AGSO Standard
C28 βα 20S
C28 βα 20R
C28 ααα 20S
C28 αββ 20R
C28 αββ 20S
C28 ααα 20R
C28
386 → 217 Da
5.2
100 %
90
80
70
60
50
40
30
20
10
0
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

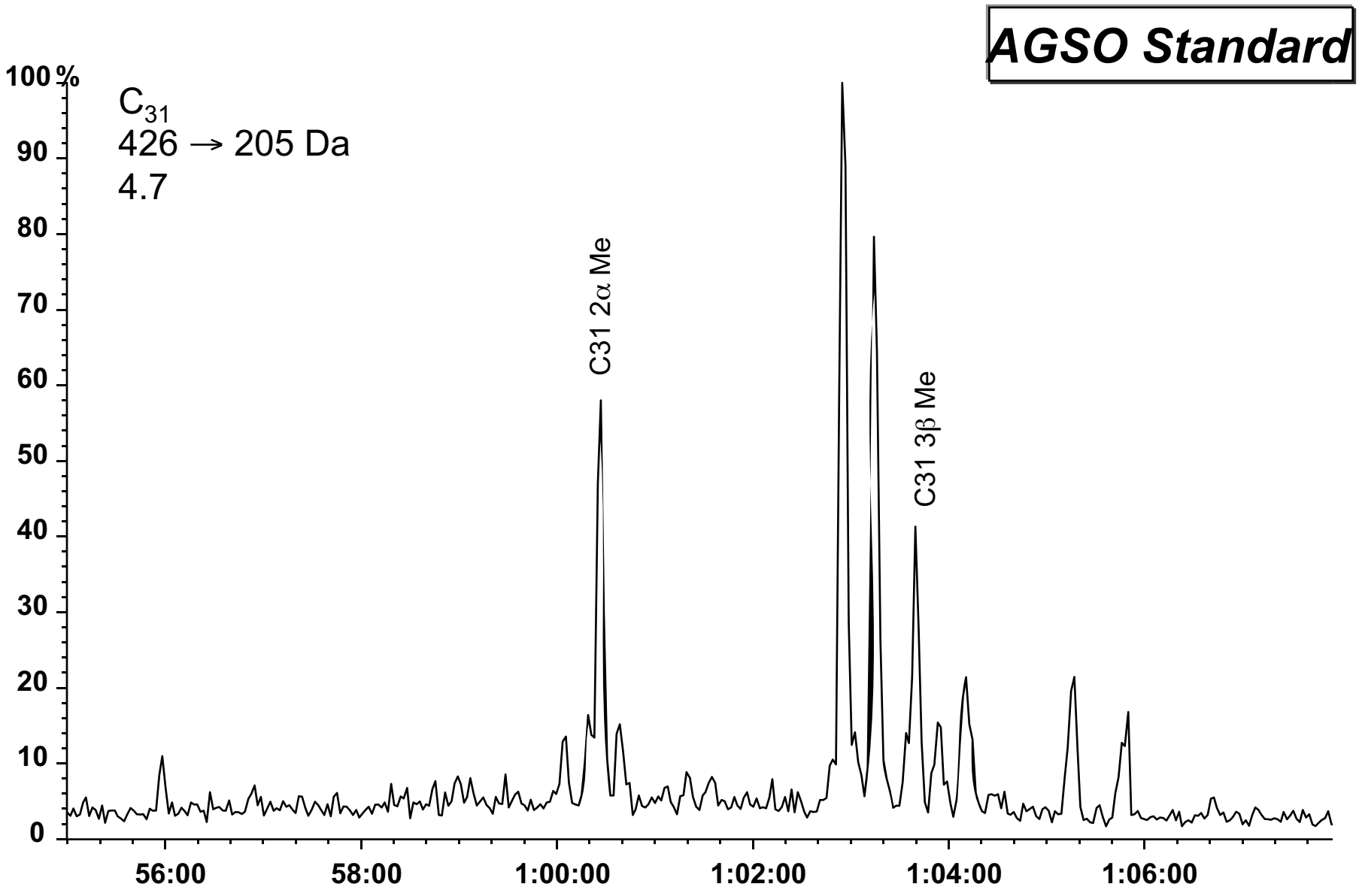

AGSO Standard
GCMS Internal Standard
C_{29}
404 → 221 Da
13.4
D_4-ααα-stigmastane 20R
100 %
90
80
70
60
50
40
30
20
10
0
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

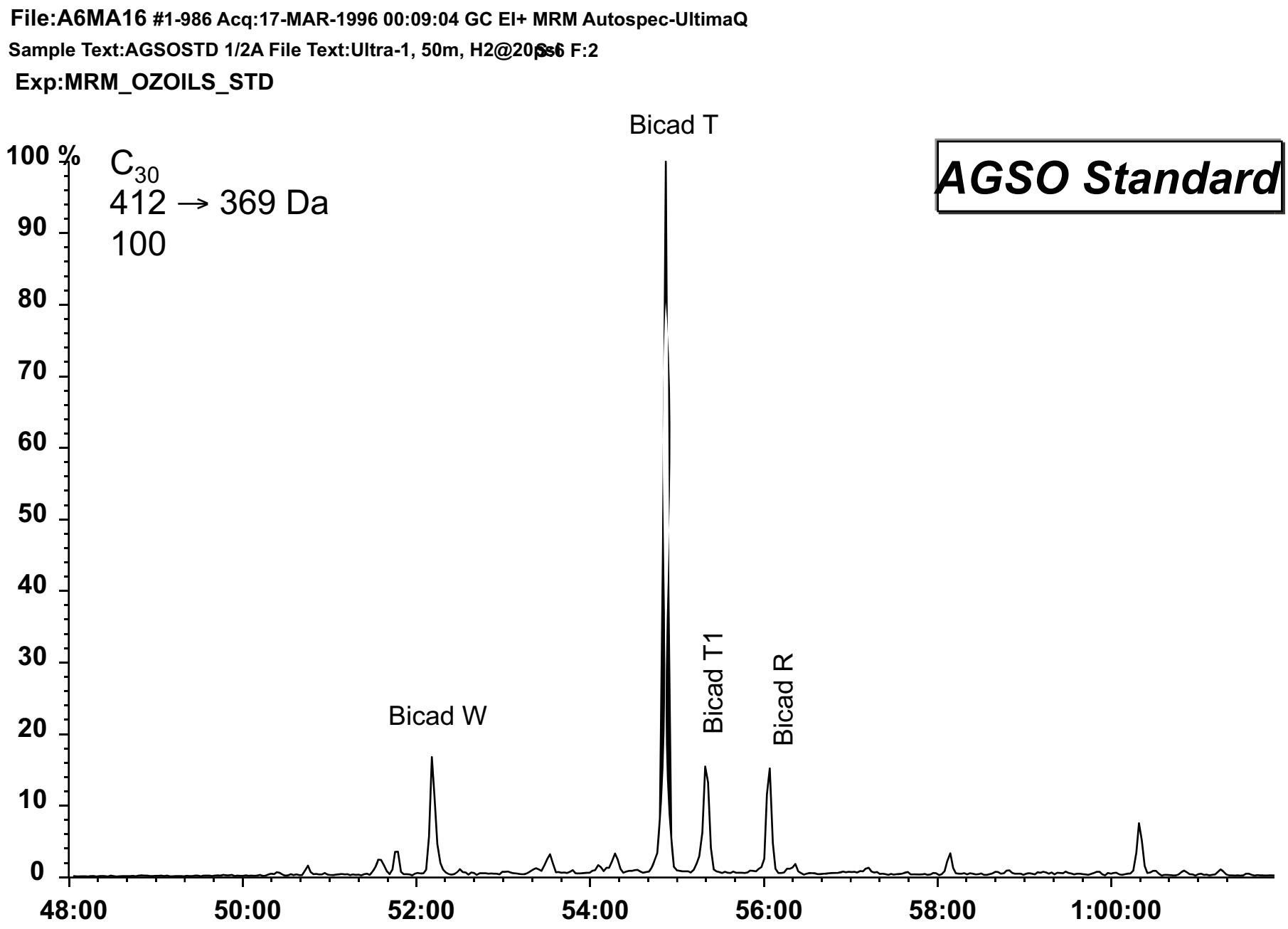

AGSO Standard
100 %
90
80
70
60
50
40
30
20
10
0
C29
400 → 217 Da
13.4
C29 βα 20S
C29 βα 20R
C29 ααα 20S
C29 αββ 20R
C29 αββ 20S
C29 ααα 20R
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

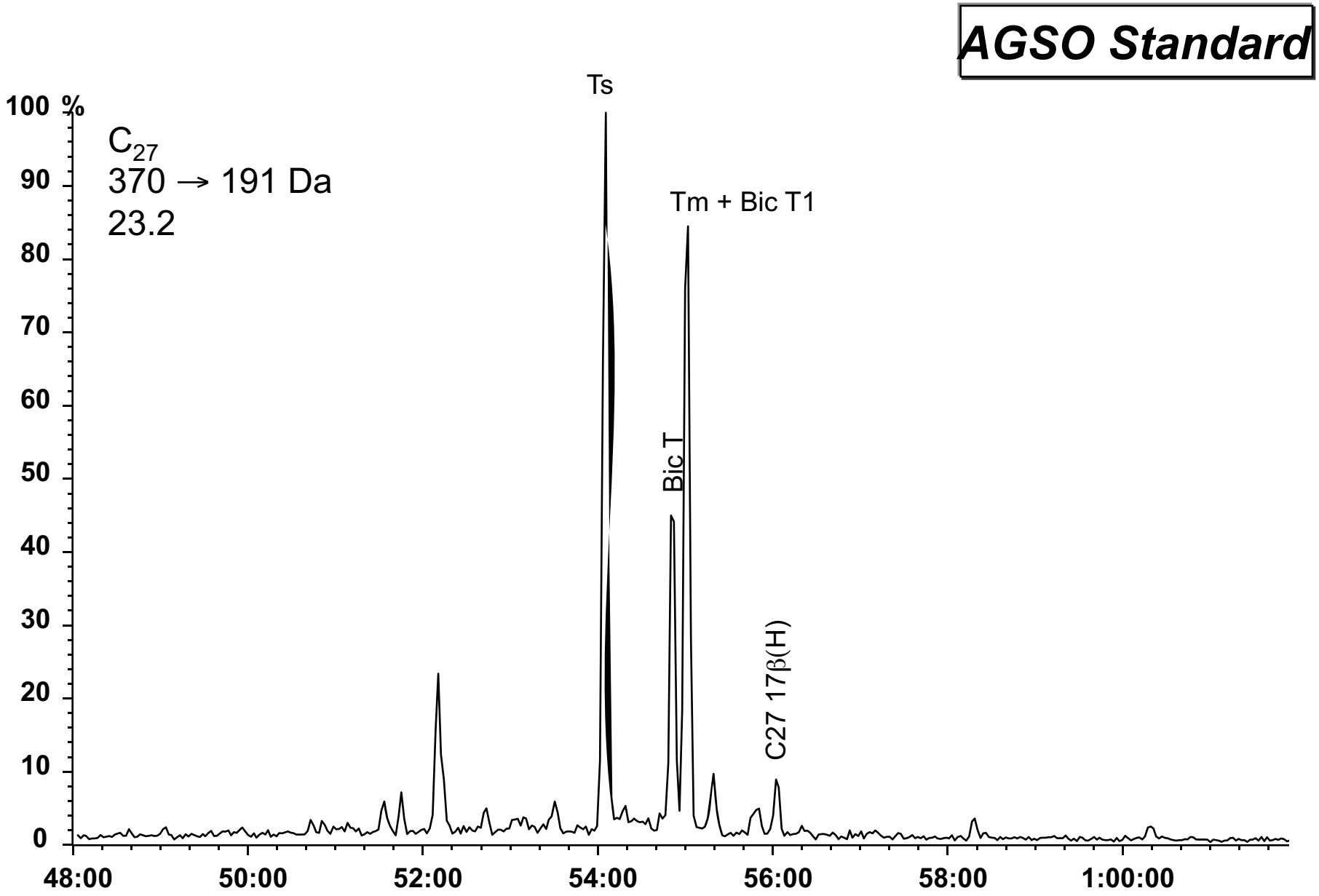

AGSO Standard
100 %
90
80
70
60
50
40
30
20
10
0
C30
414 → 217 Da
0.9
C30 βα 20S
C30 βα 20R
C30 ααα 20S
C30 αββ 20(R+S)
C30 ααα 20R
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

AGSO Standard

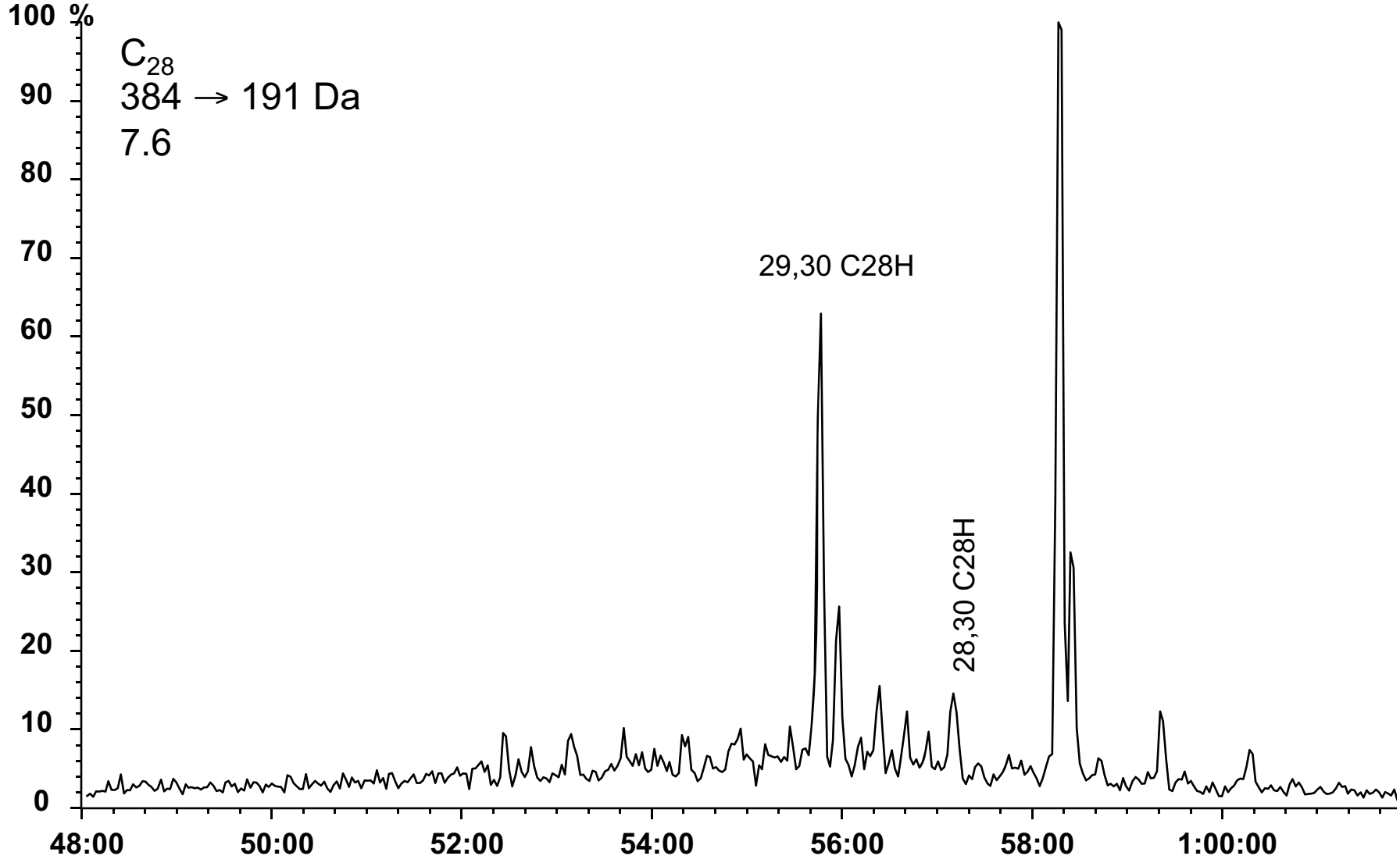



AGSO Standard
100 %
90
80
70
60
50
40
30
20
10
0
C31
426 → 205 Da
4.7
C31 2α Me
C31 3β Me
56:00
58:00
1:00:00
1:02:00
1:04:00
1:06:00

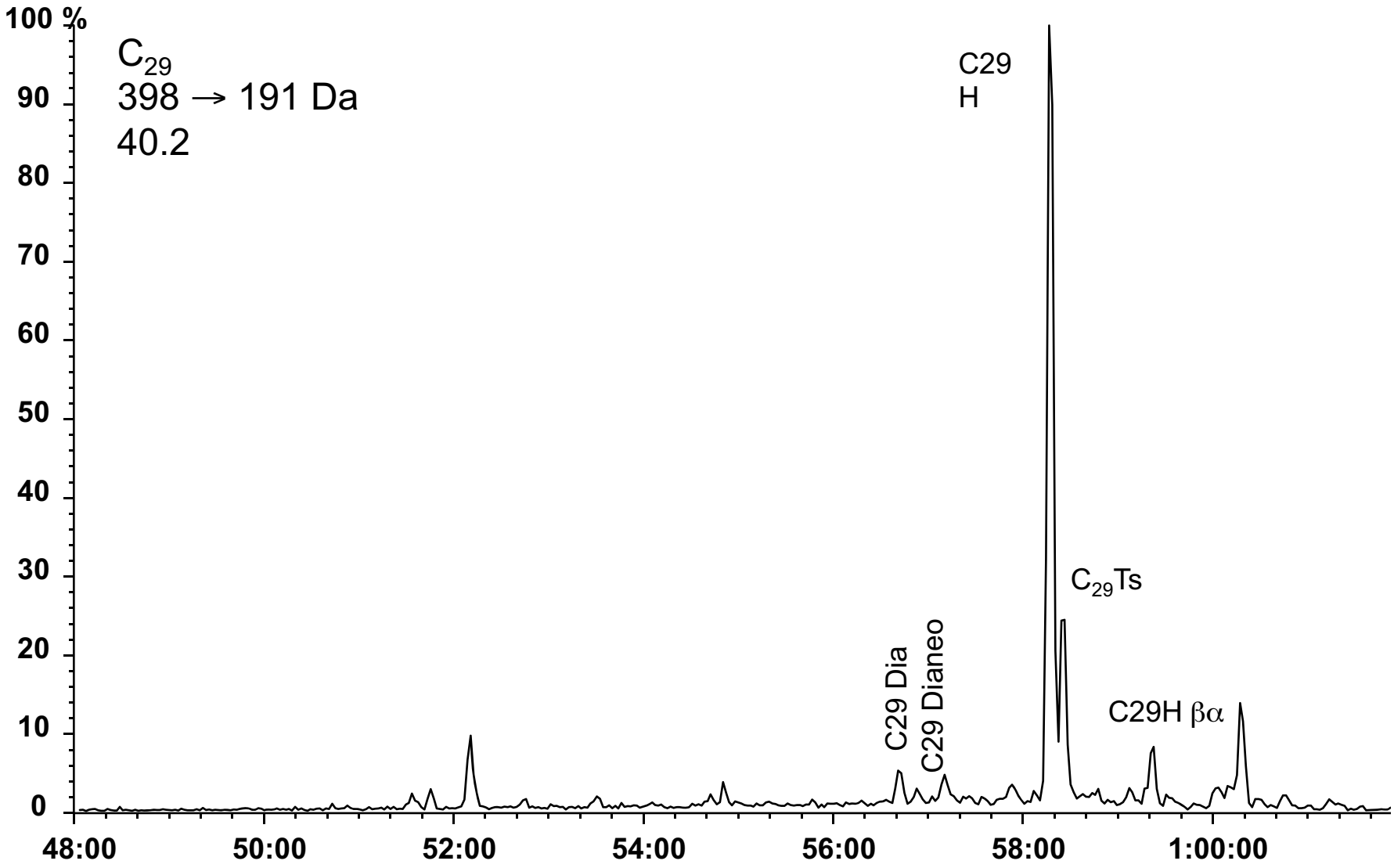

File:A6MA16 #1-986 Acq:17-MAR-1996 00:09:04 GC EI+ MRM Autospec-UltimaQ
Sample Text:AGSOSTD 1/2A File Text:Ultra-1, 50m, H2@20psi F:2
Exp:MRM_OZOILS_STD
Bicad T
100 %
C30
412 → 369 Da
100
AGSO Standard
90
80
70
60
50
40
30
20
10
0
Bicad W
Bicad T1
Bicad R
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

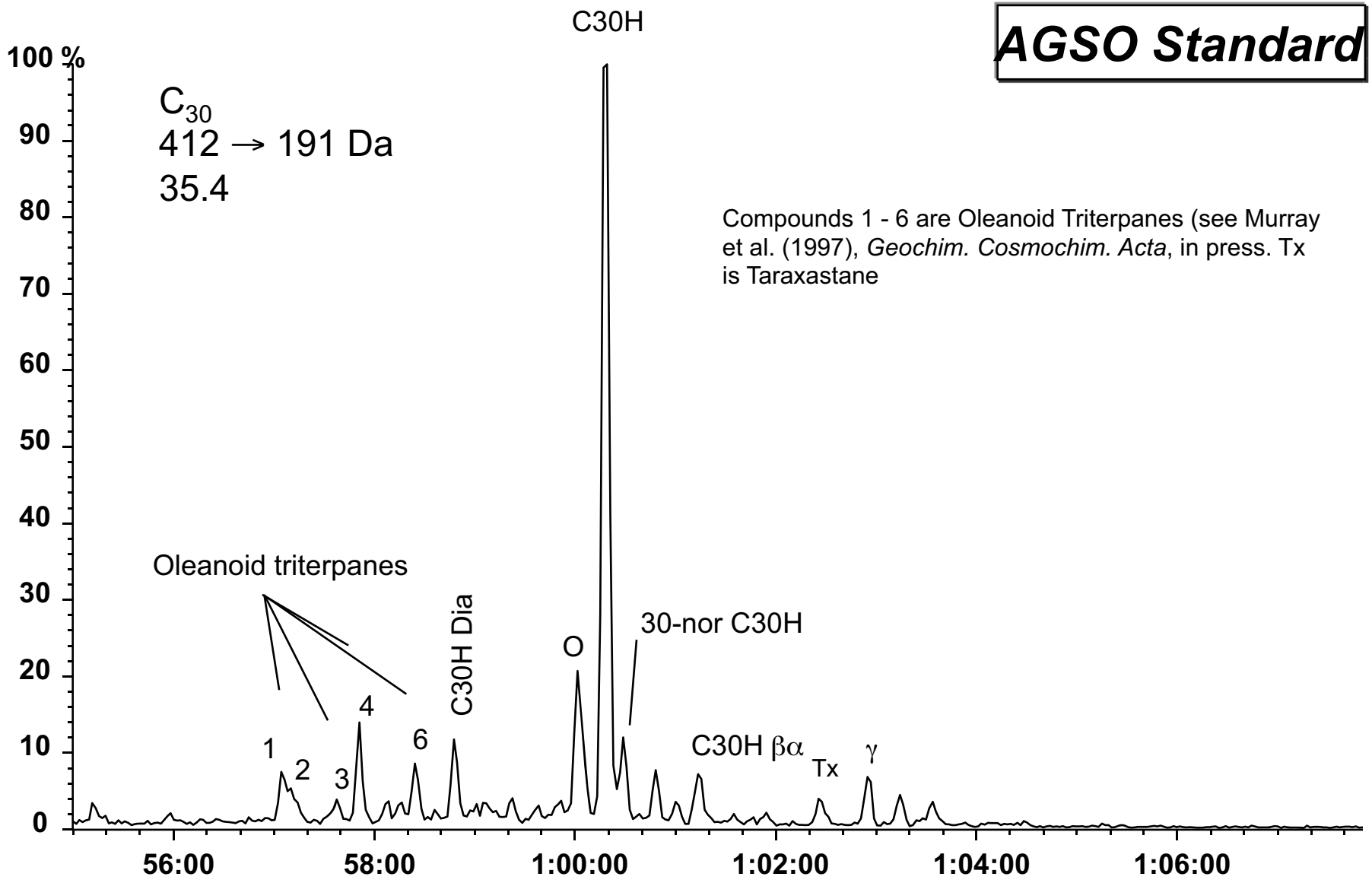

AGSO Standard
Ts
100 %
C27
370 → 191 Da
23.2
Tm + Bic T1
90
80
70
60
50
40
Bic T
30
20
C27 17β(H)
10
0
48:00
50:00
52:00
54:00
56:00
58:00
1:00:00

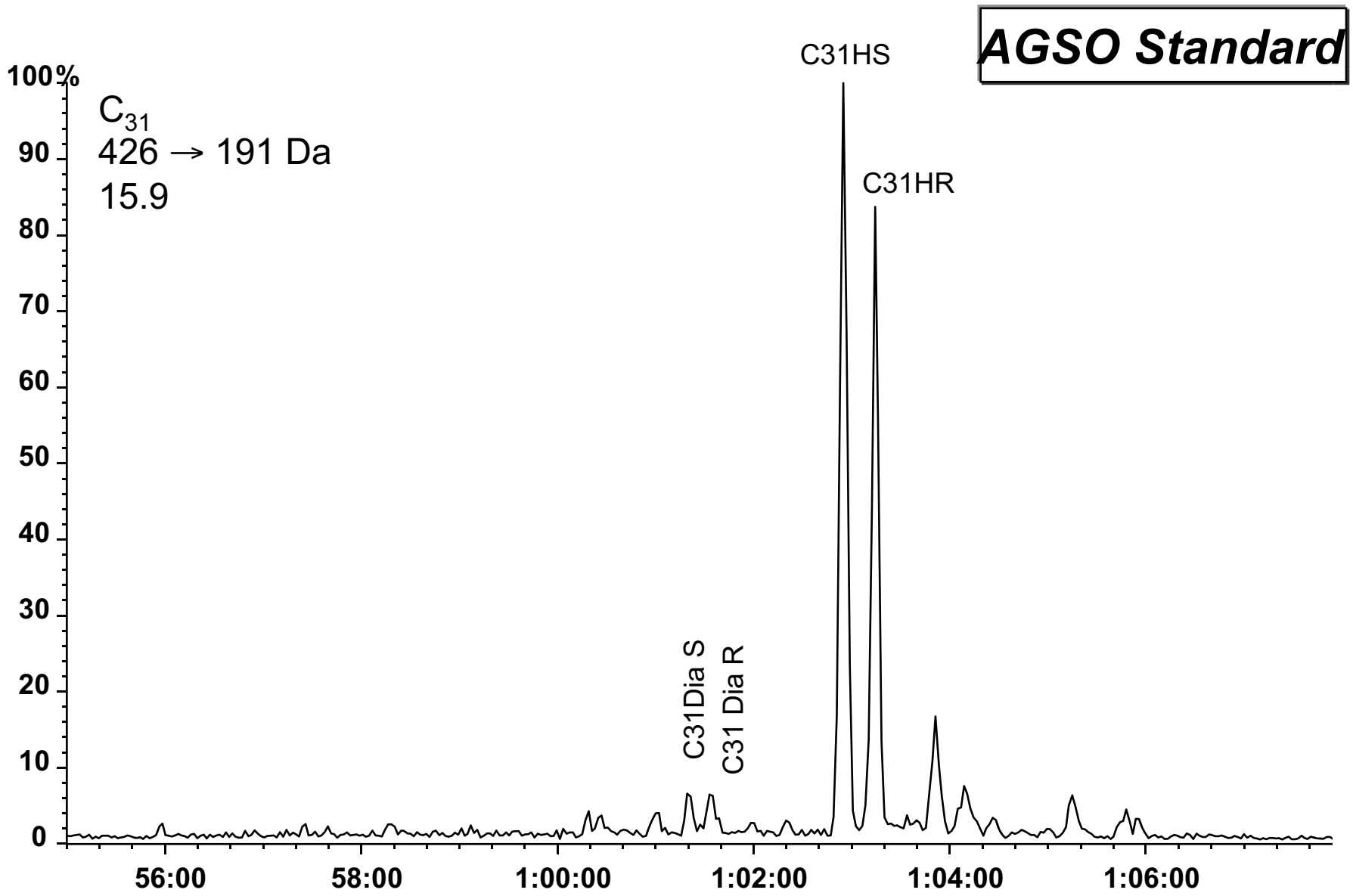
AGSO Standard



AGSO Standard

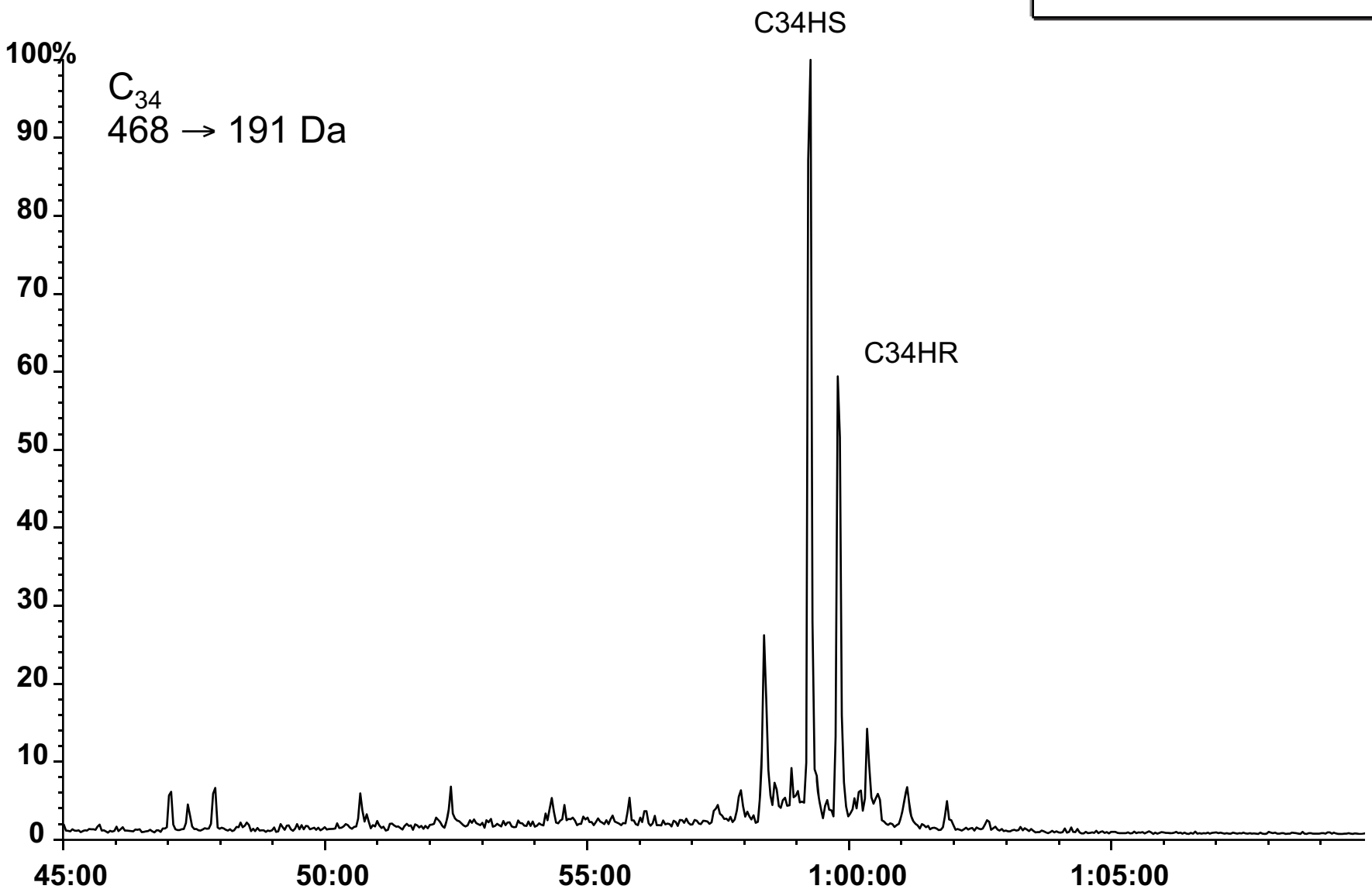



AGSO Standard
C30H
100 %
90
80
70
60
50
40
30
20
10
0
C30
412 → 191 Da
35.4
Compounds 1 - 6 are Oleanoid Triterpanes (see Murray et al. (1997), *Geochim. Cosmochim. Acta*, in press. Tx is Taraxastane
Oleanoid triterpanes
1
2
3
4
6
C30H Dia
O
30-nor C30H
C30H βα
Tx
γ
56:00
58:00
1:00:00
1:02:00
1:04:00
1:06:00


AGSO Standard
C31HS
C31HR
C31Dia S
C31 Dia R
C_{31}
426 → 191 Da
15.9
100%
90
80
70
60
50
40
30
20
10
0
56:00
58:00
1:00:00
1:02:00
1:04:00
1:06:00

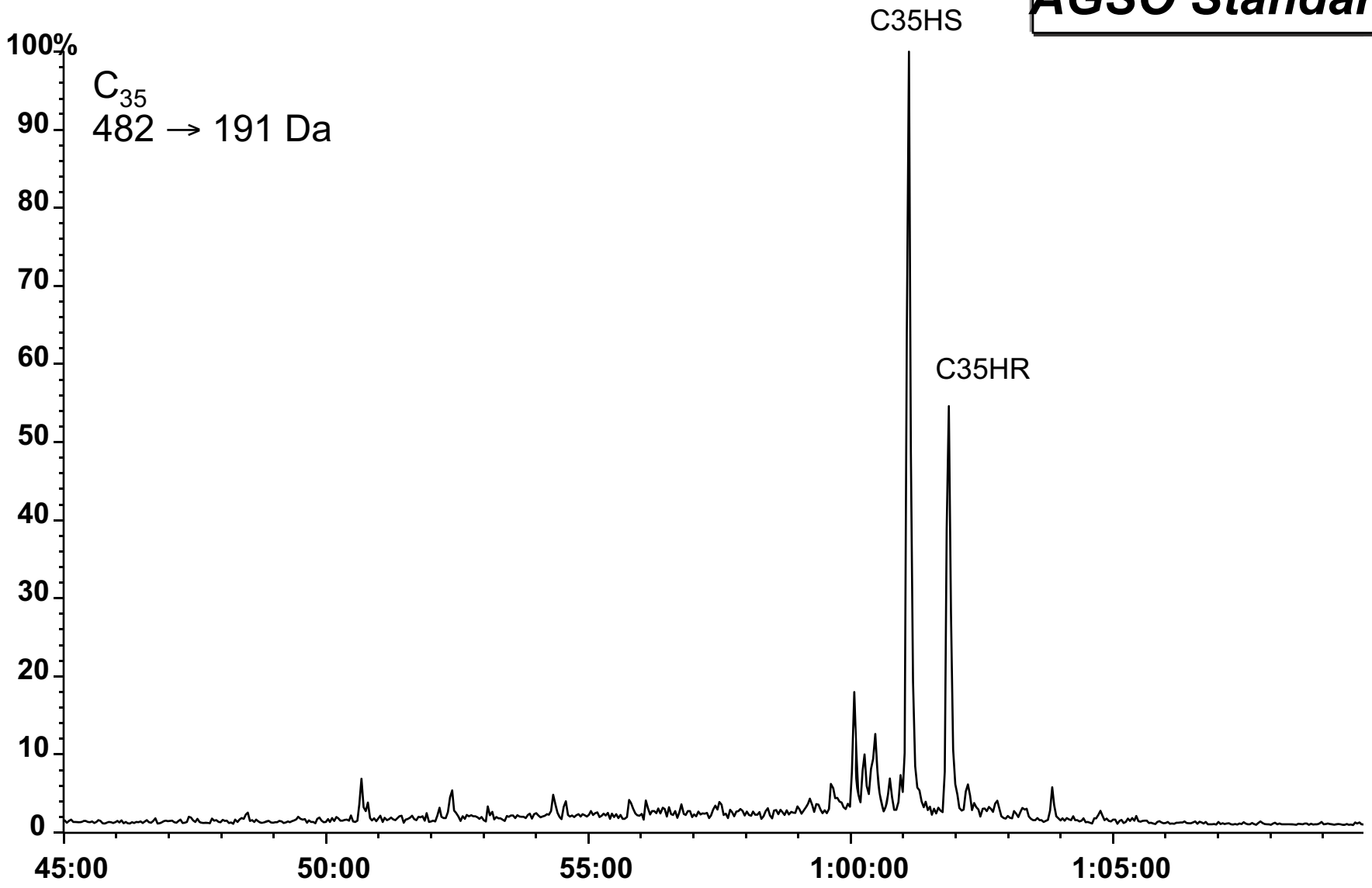
Note: This trace is from run A6FE24:5
using MRM-OZOILS_50

AGSO Standard



Note: This trace is from run A6FE24:5
using MRM-OZOILS_50

AGSO Standard



12.158 Molecular Biogeochemistry

Fall 2011