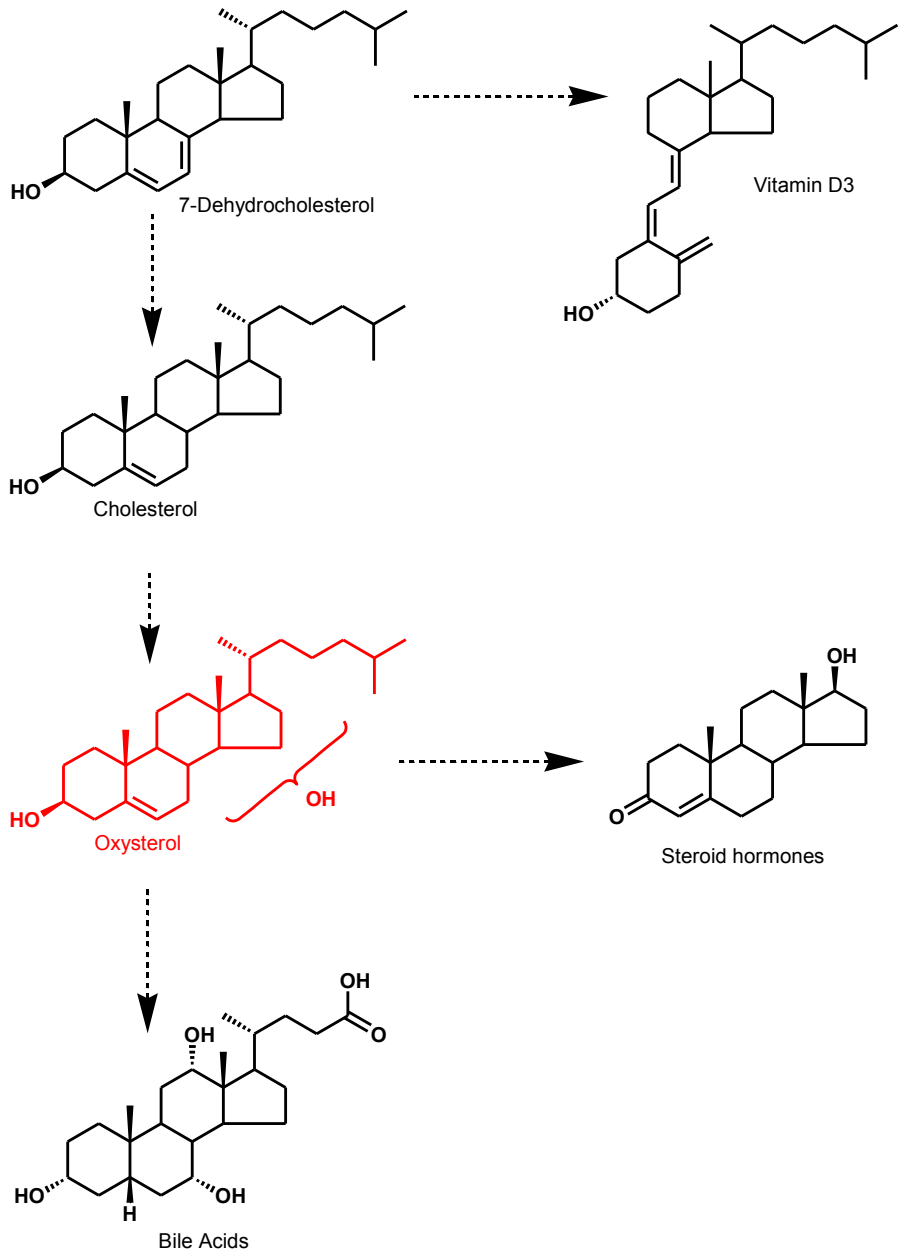
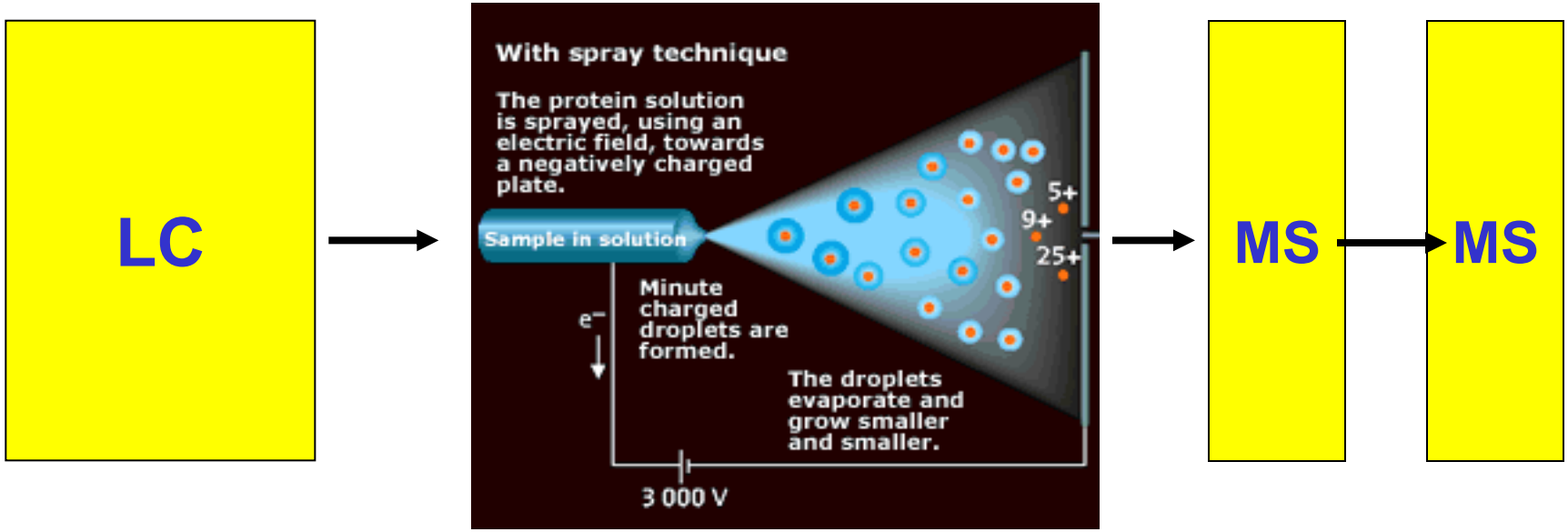
LC-MS from bench to bedside

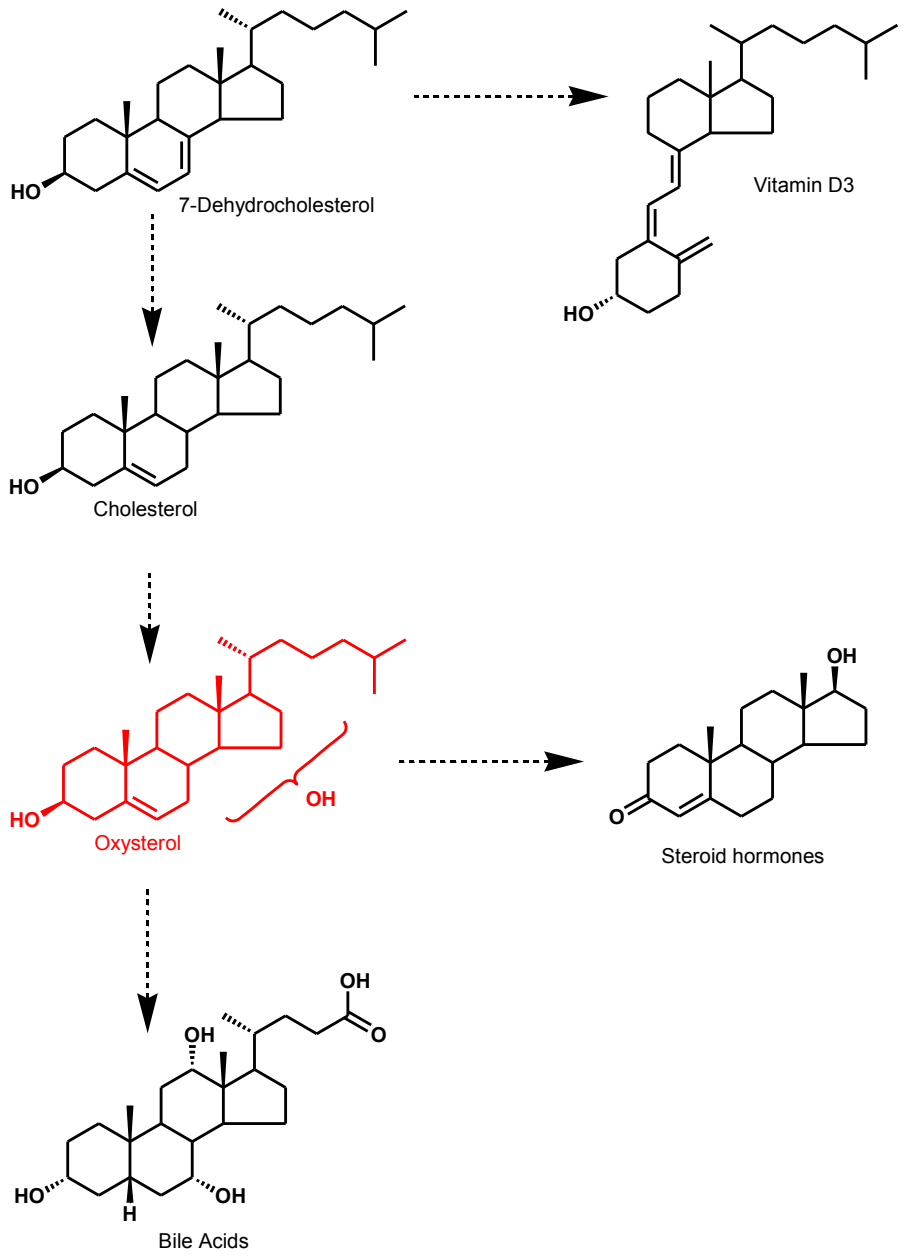
William J. Griffiths
Swansea University


HO
7-Dehydrocholesterol
Vitamin D3
HO
HO
Cholesterol
HO
OH
Oxysterol
OH
O
Steroid hormones
OH
O
OH
HO
OH
H
Bile Acids

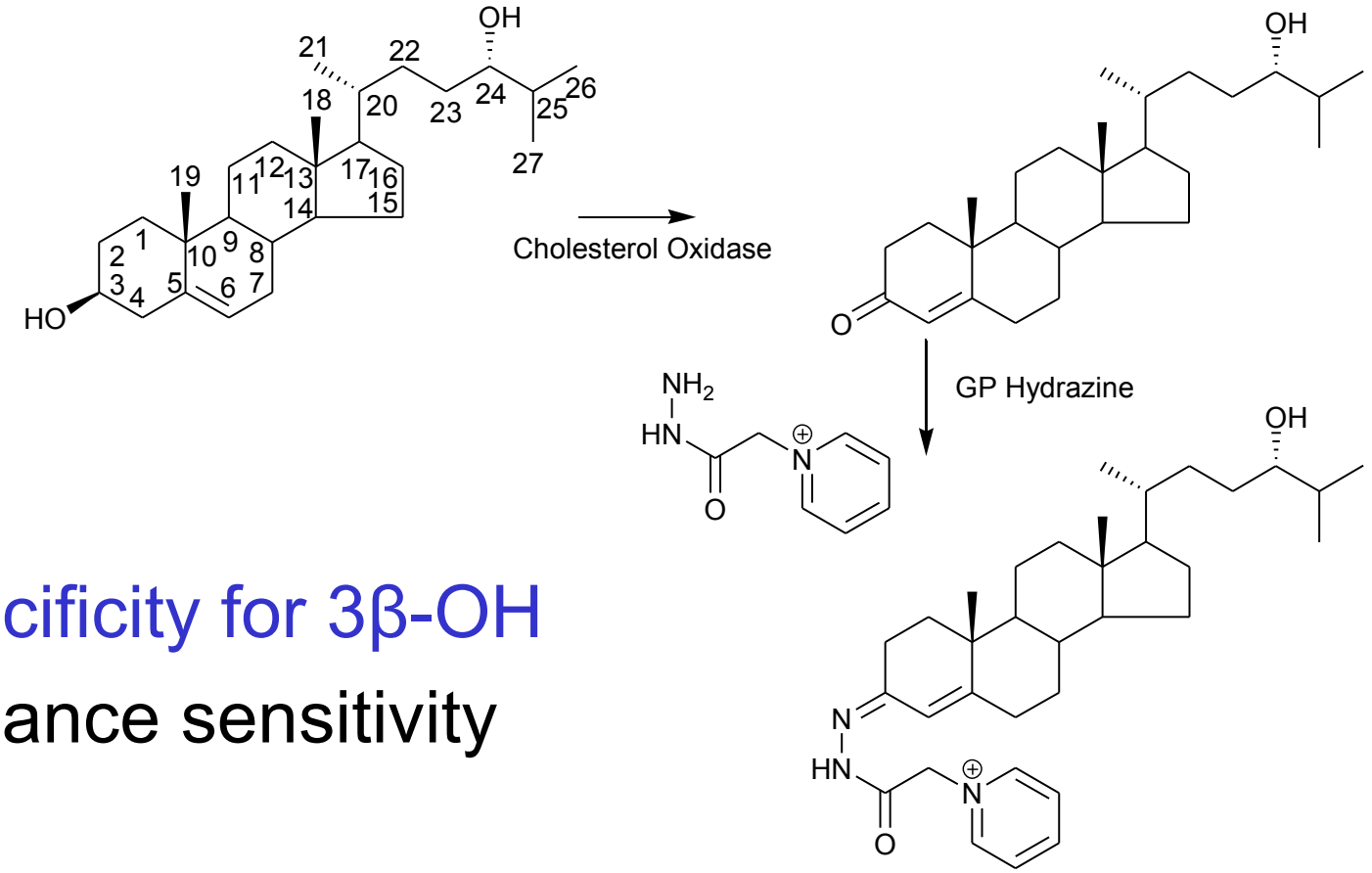
“Steroidomics”



LC-ESI-MS/MS


HO
7-Dehydrocholesterol
Vitamin D3
HO
HO
Cholesterol
HO
OH
Oxysterol
OH
O
Steroid hormones
OH
O
OH
HO
OH
H
Bile Acids

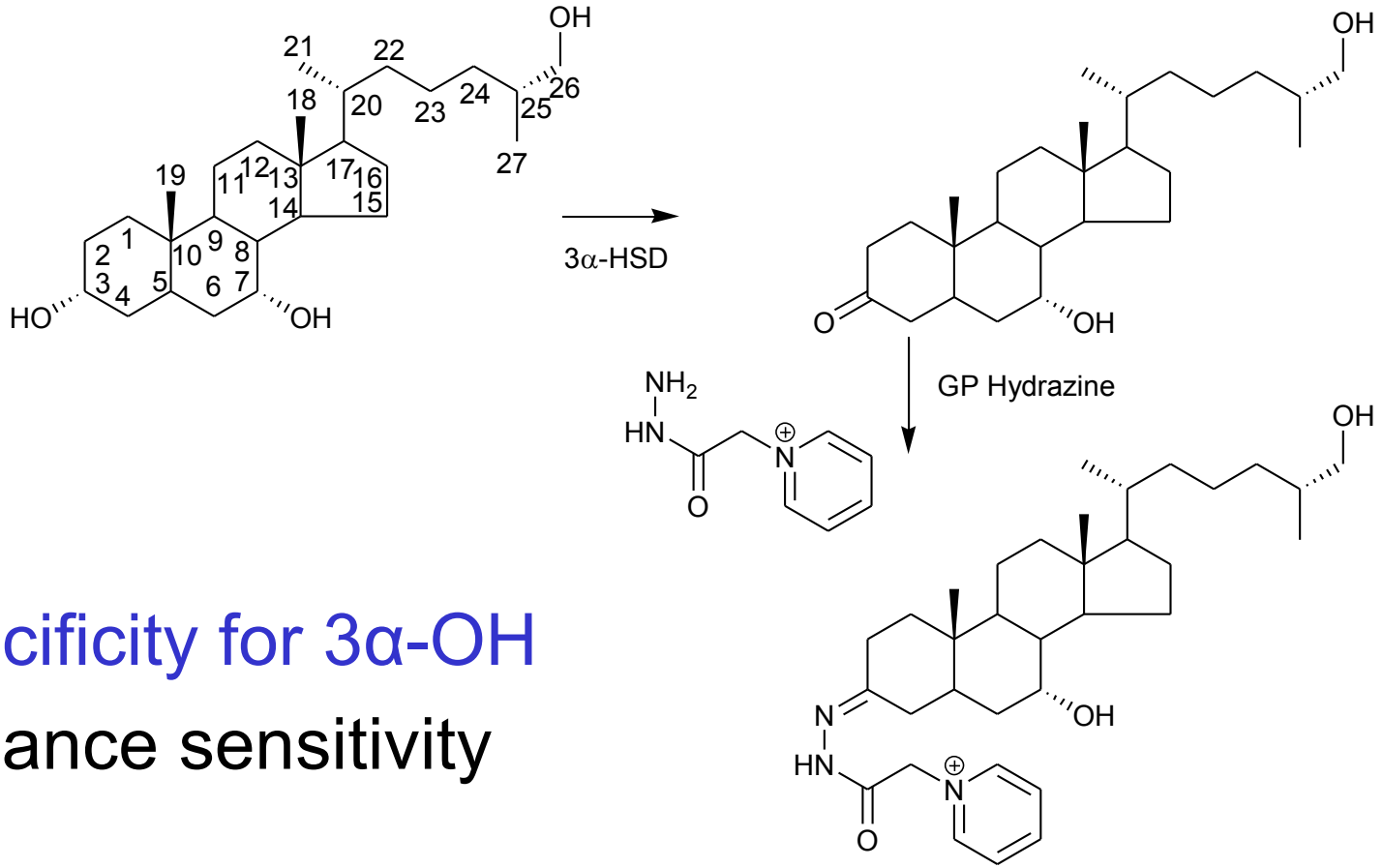
Enzyme-Assisted Derivatisation for Sterol Analysis (EADSA)



- Specificity for 3β-OH
- Enhance sensitivity

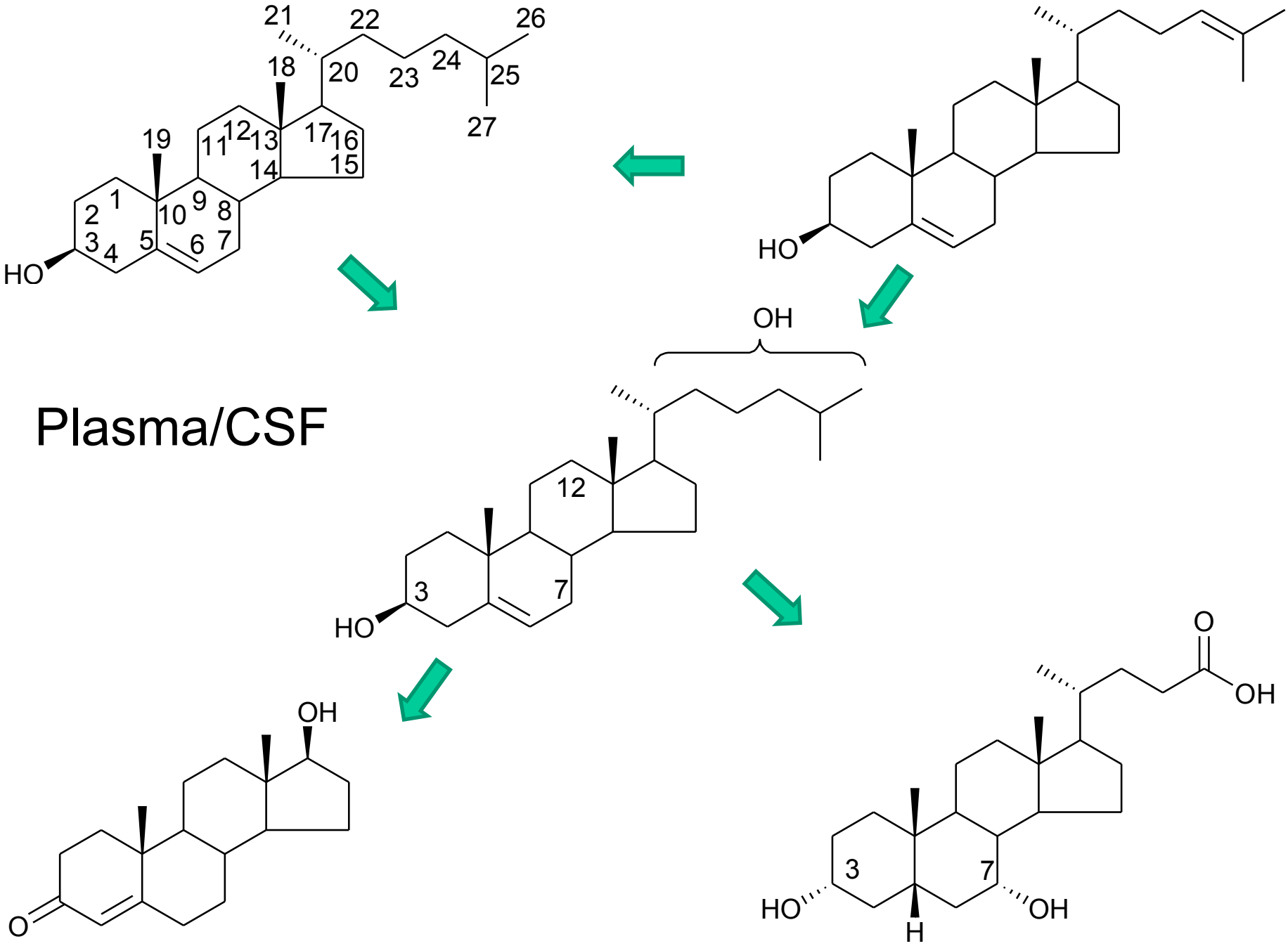
Griffiths et al, RCM 2003 17 924
Shackleton et al, Steroids 1997 62 523
Smith & Brooks, J Chromatogr 1974 101 373
Girard & Sandulesco, Helv Chim Acta 1936 19 1095

Enzyme-Assisted Derivatisation for Sterol Analysis (EADSA)

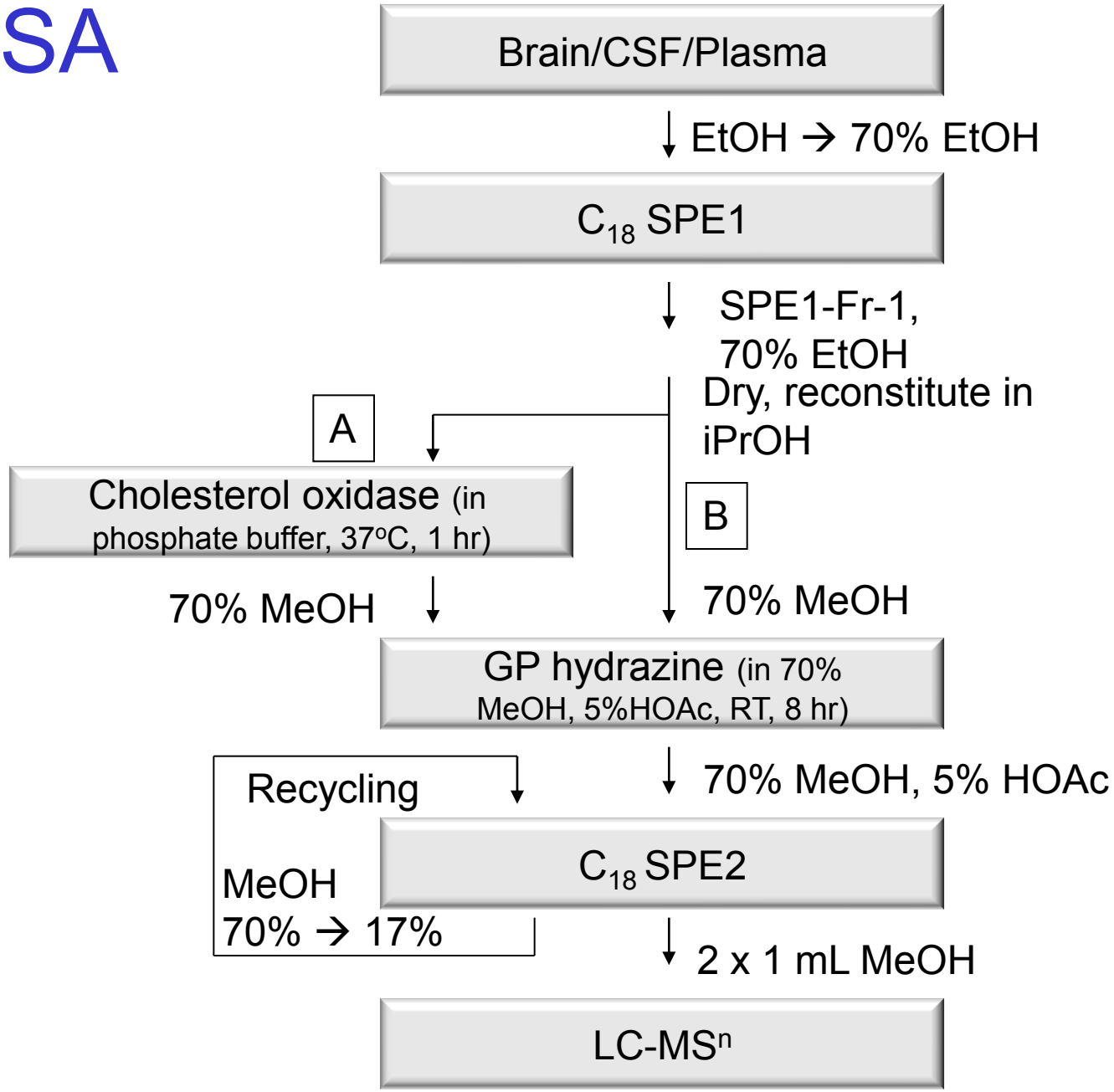


- Specificity for 3α-OH
- Enhance sensitivity

Griffiths et al, RCM 2003 17 924
Shackleton et al, Steroids 1997 62 523
Smith & Brooks, J Chromatogr 1974 101 373
Girard & Sandulesco, Helv Chim Acta 1936 19 1095

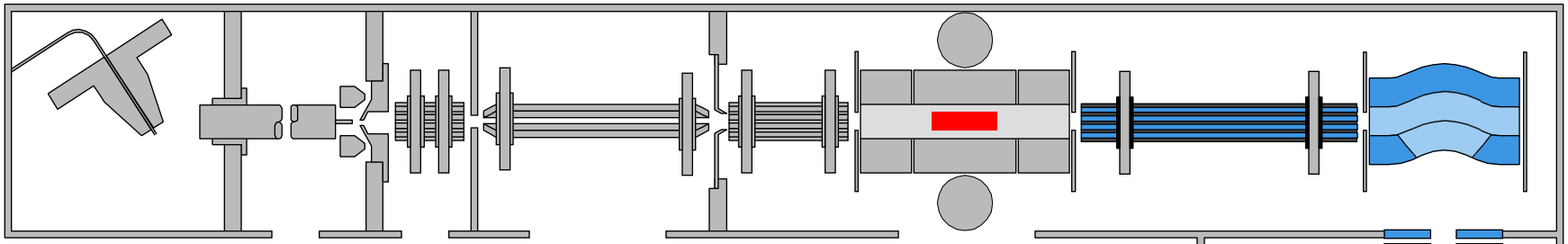

Plasma/CSF
HO
OH
O
OH
HO
OH
H

EADSA

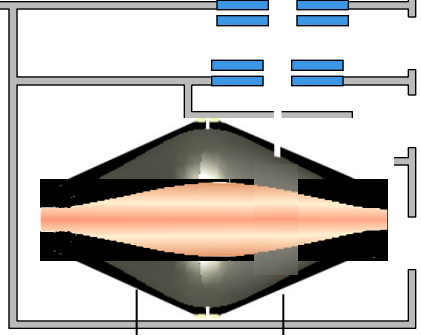

Brain/CSF/Plasma
EtOH → 70% EtOH
C18 SPE1
SPE1-Fr-1, 70% EtOH
Dry, reconstitute in iPrOH
A
B
Cholesterol oxidase (in phosphate buffer, 37°C, 1 hr)
70% MeOH
70% MeOH
GP hydrazine (in 70% MeOH, 5%HOAc, RT, 8 hr)
70% MeOH, 5% HOAc
Recycling
MeOH 70% → 17%
C18 SPE2
2 x 1 mL MeOH
LC-MSn

LTQ Orbitrap Operation Principle

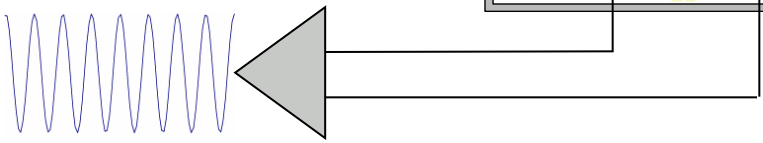
1. Ions are stored in the Linear Trap
2. …. are axially ejected
3. …. and trapped in the C-trap
4. …. they are squeezed into a small cloud and injected into the Orbitrap
5. …. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation

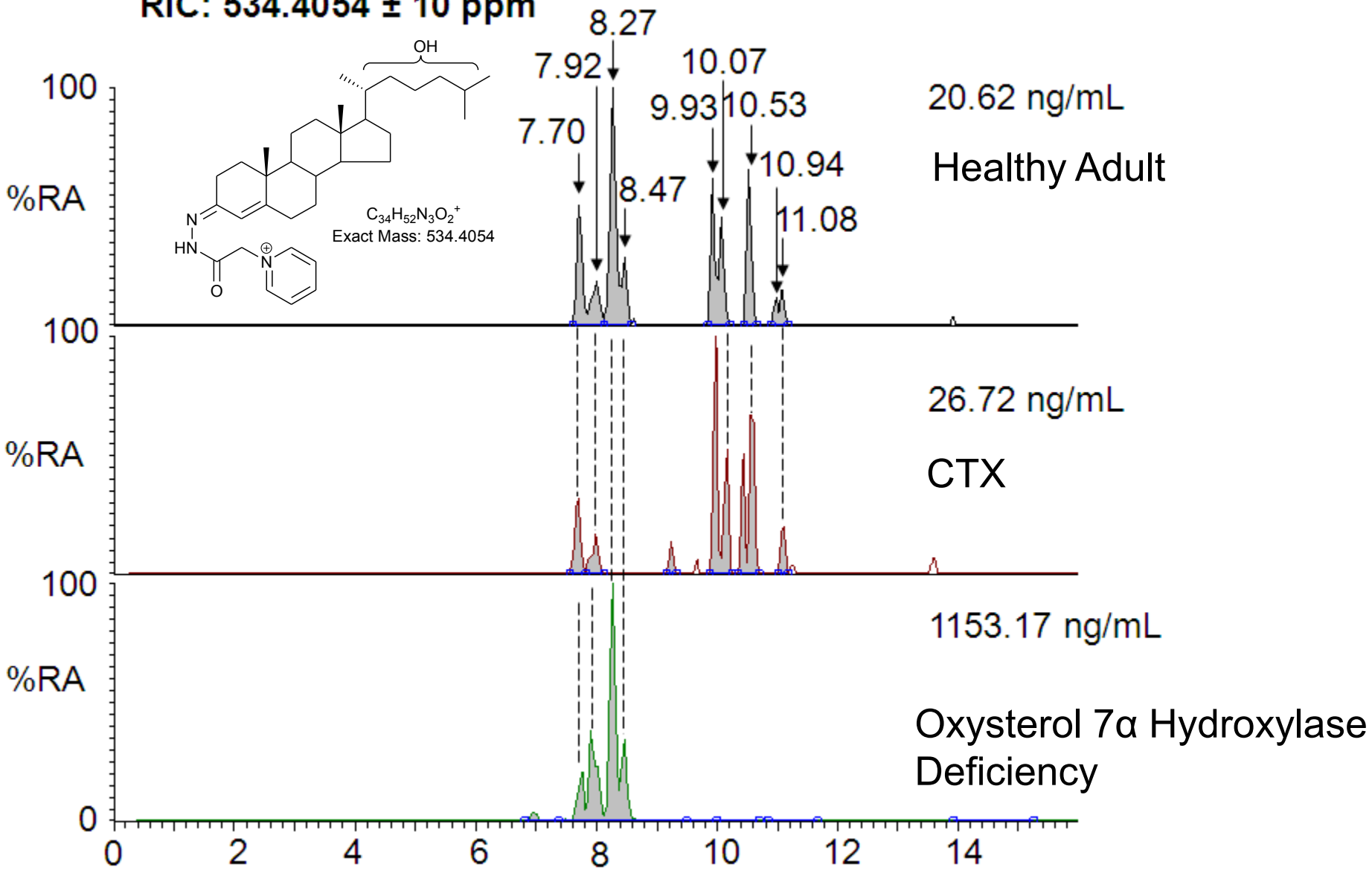


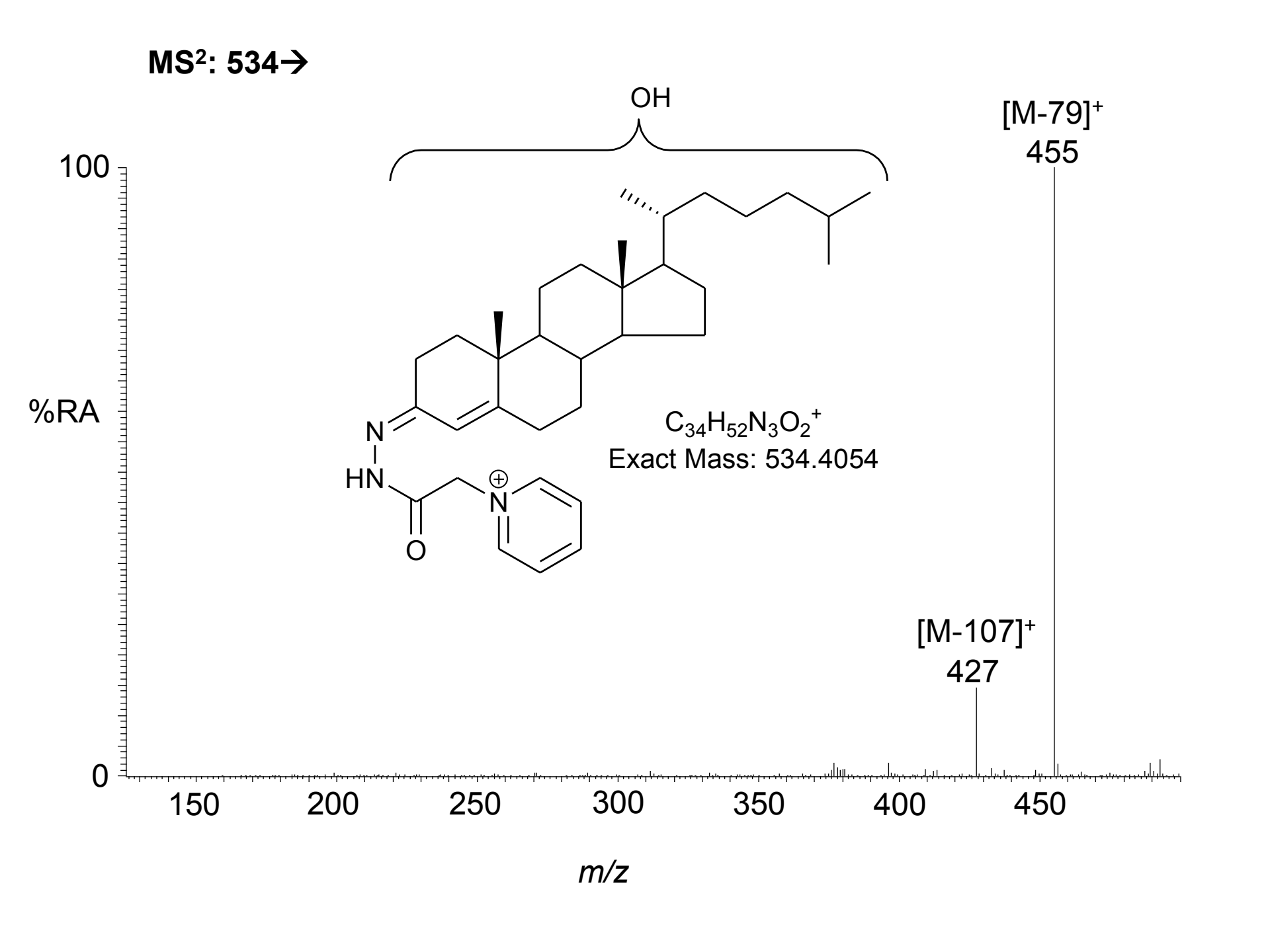
The oscillating ions induce an image current into the two outer halves of the orbitrap, which can be detected using a differential amplifier

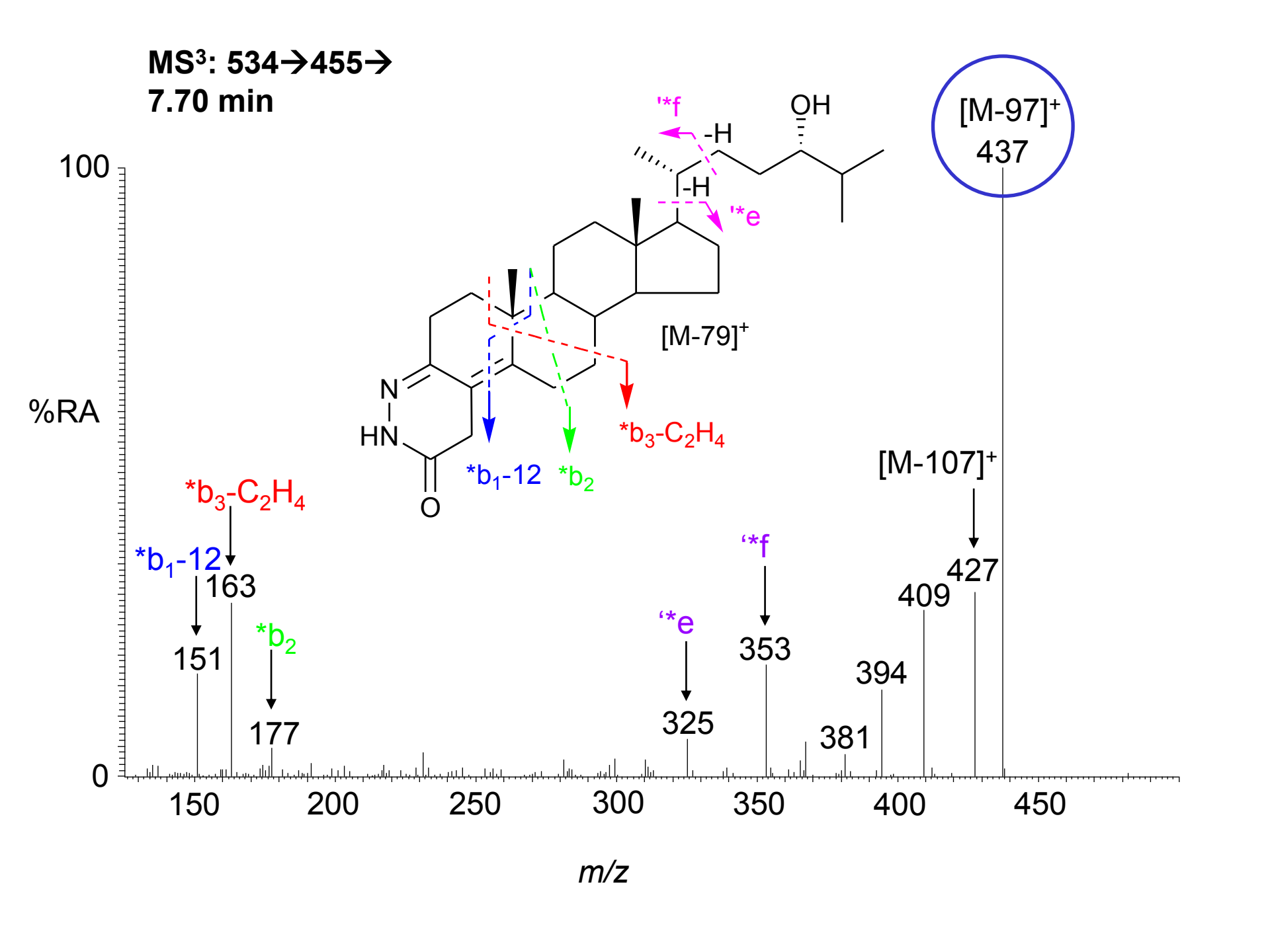


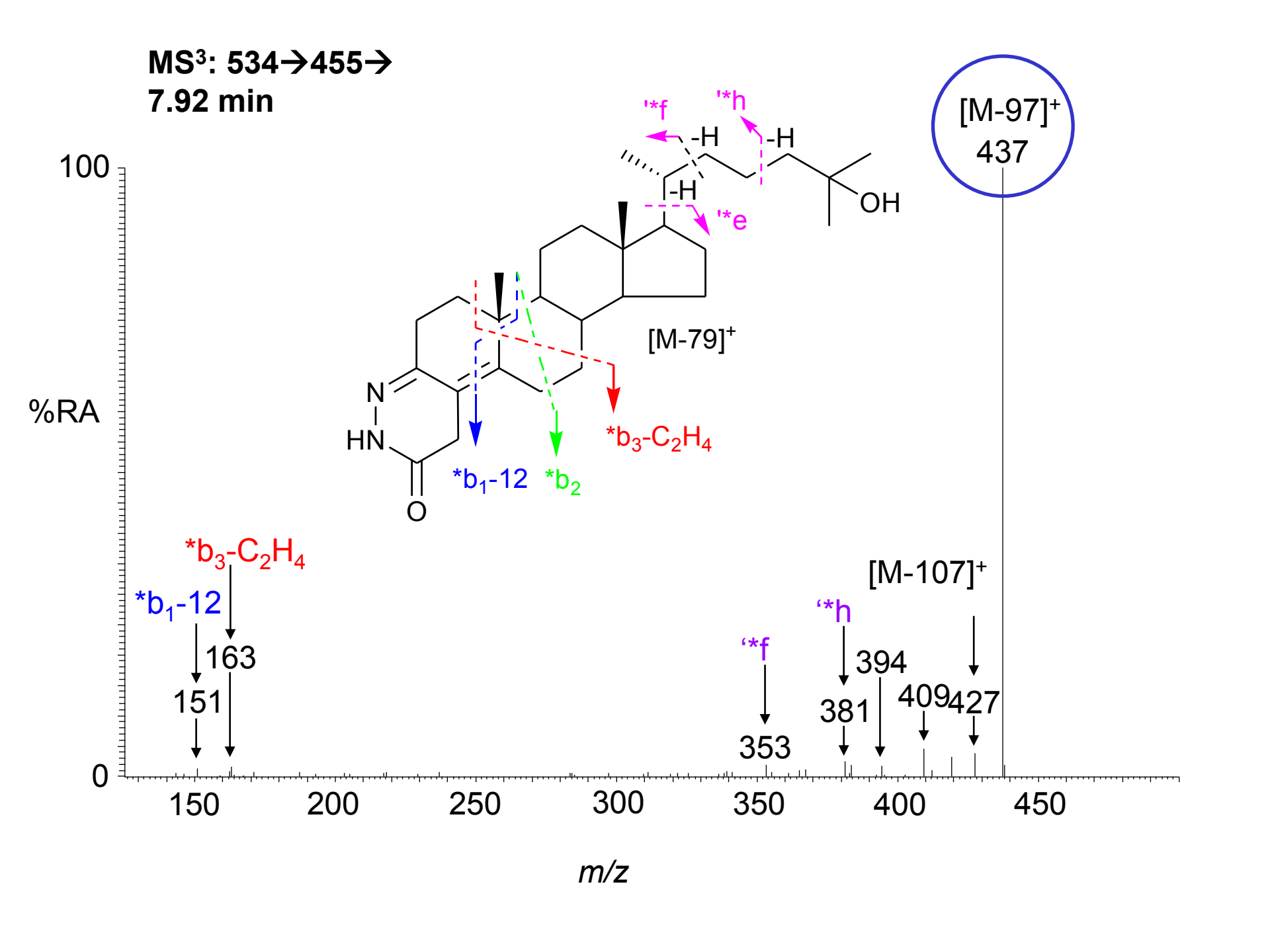
Ions of only one mass generate a sine wave signal

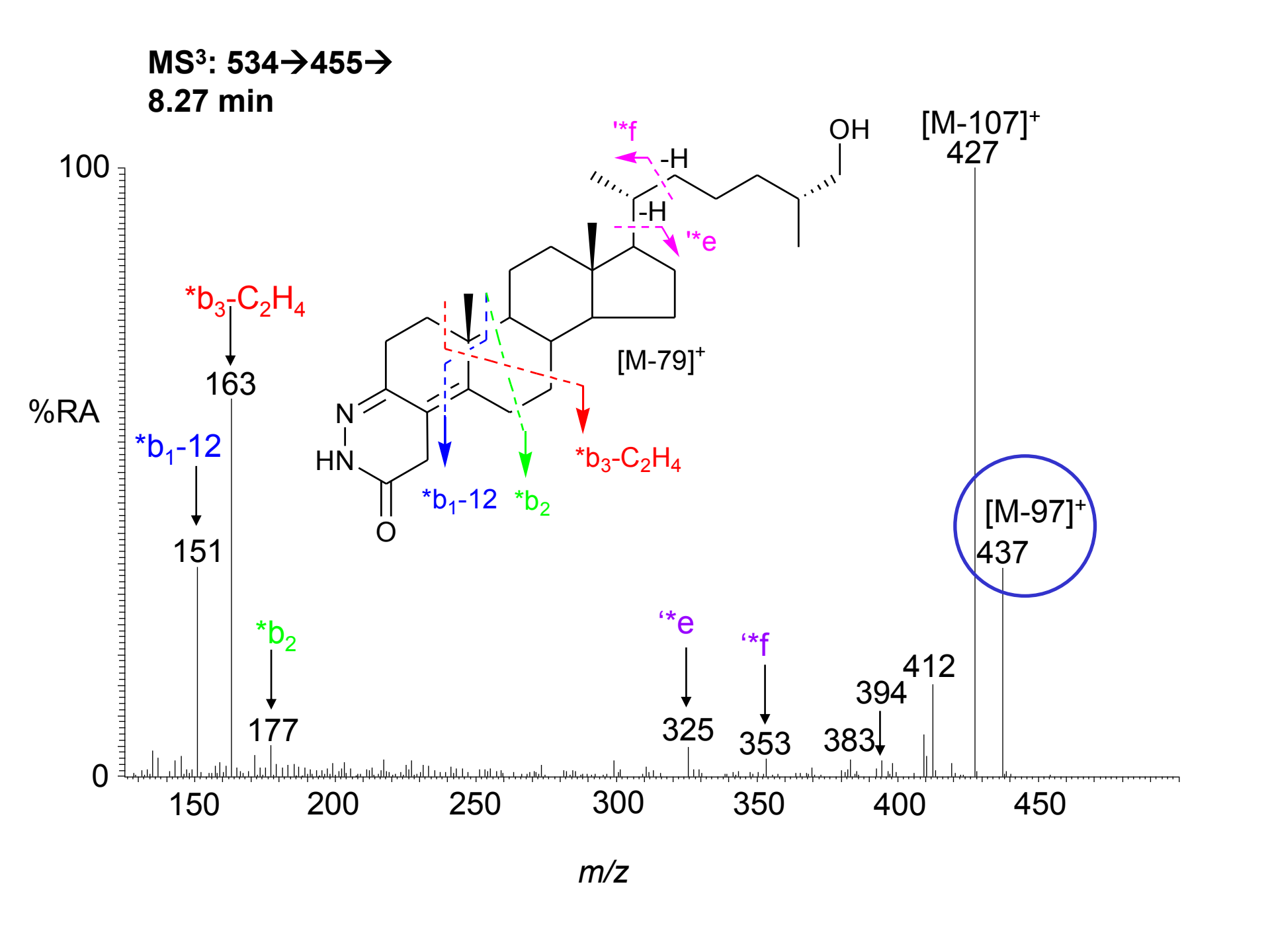


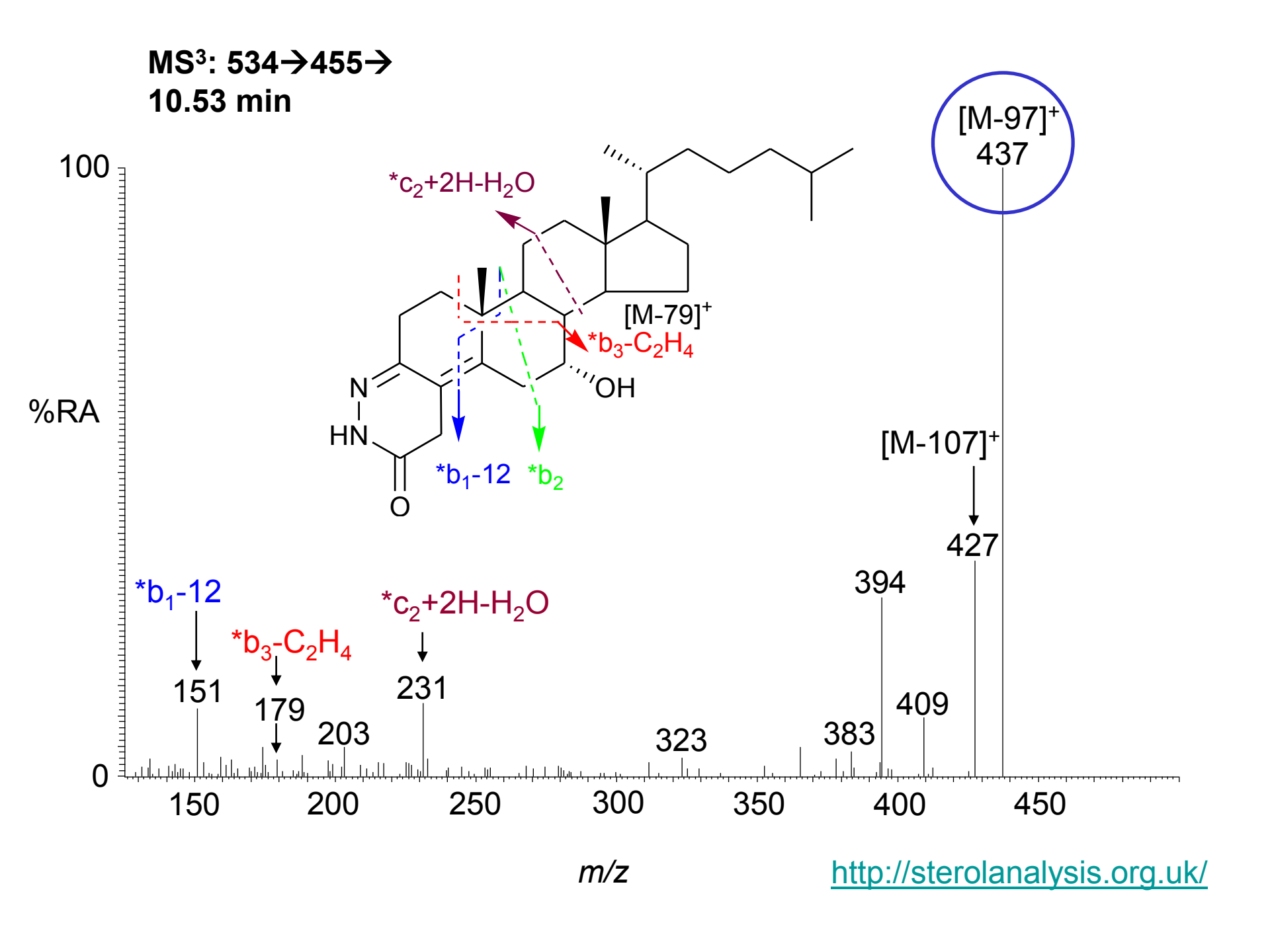

RIC: 534.4054 ± 10 ppm
OH
N
HN
O
N
$C_{34}H_{52}N_3O_2^+$
Exact Mass: 534.4054
8.27
7.92
10.07
9.93
10.53
7.70
10.94
8.47
11.08
100
%RA
20.62 ng/mL
Healthy Adult
100
%RA
26.72 ng/mL
CTX
100
%RA
0
1153.17 ng/mL
Oxysterol 7α Hydroxylase Deficiency
0
2
4
6
8
10
12
14

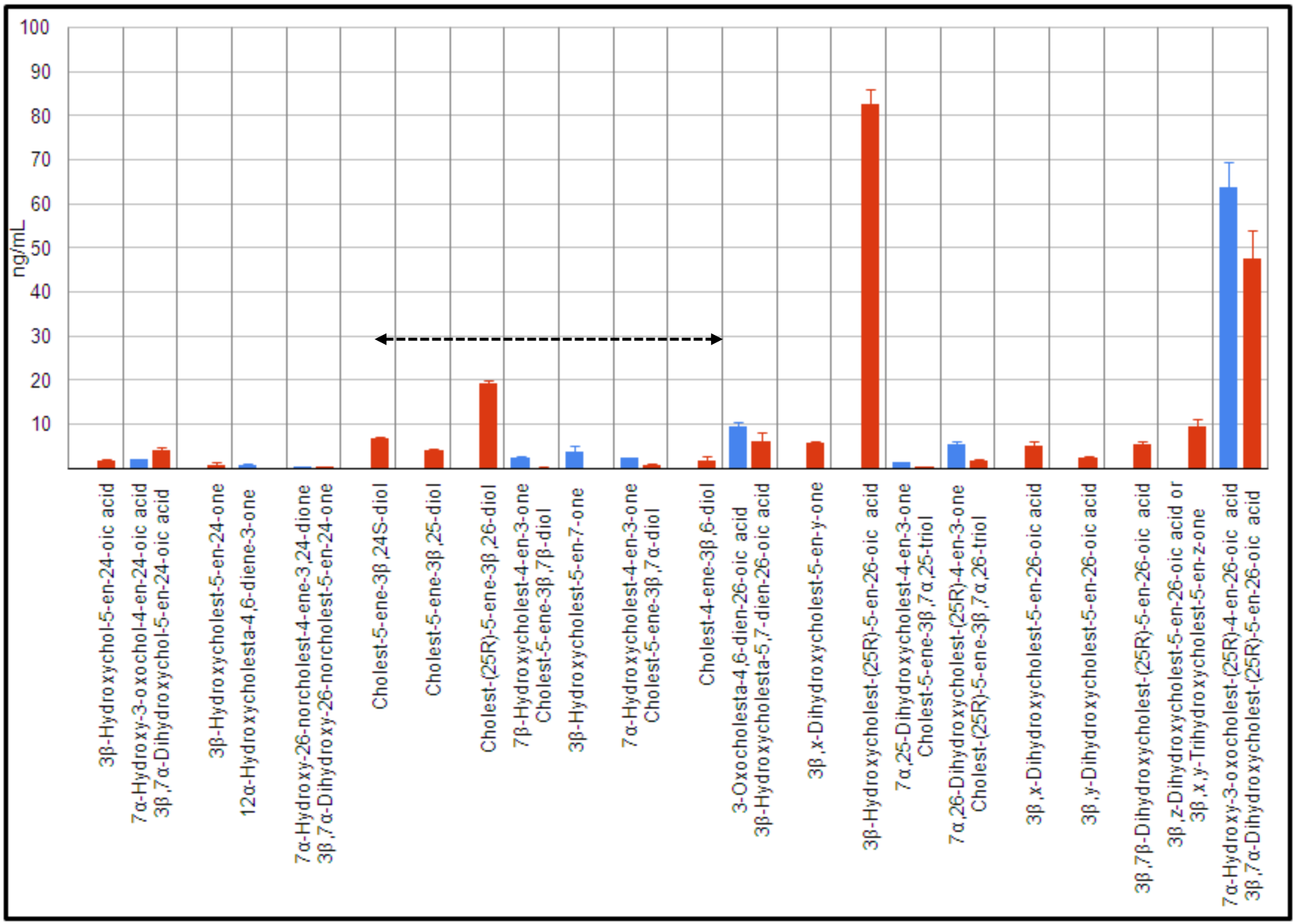

MS²: 534→
OH
[M-79]⁺
455
100
%RA
0
N
HN
O
N
C34H52N3O2+
Exact Mass: 534.4054
[M-107]⁺
427
150
200
250
300
350
400
450
m/z


MS3: 534→455→
7.70 min
100
%RA
0
150
200
250
300
350
400
450
m/z
[M-97]+
437
[M-107]+
427
409
394
381
353
325
177
151
163
*b3-C2H4
*b1-12
*b2
'*f
'*e
-H
-H
OH
[M-79]+
N
HN
O


MS3: 534→455→
7.92 min
[M-97]+
437
100
%RA
0
'*f
'*h
-H
-H
-H
OH
'*e
[M-79]+
N
HN
O
*b3-C2H4
*b1-12
*b2
*b3-C2H4
*b1-12
163
151
[M-107]+
'*h
'*f
394
381
409
427
353
150
200
250
300
350
400
450
m/z

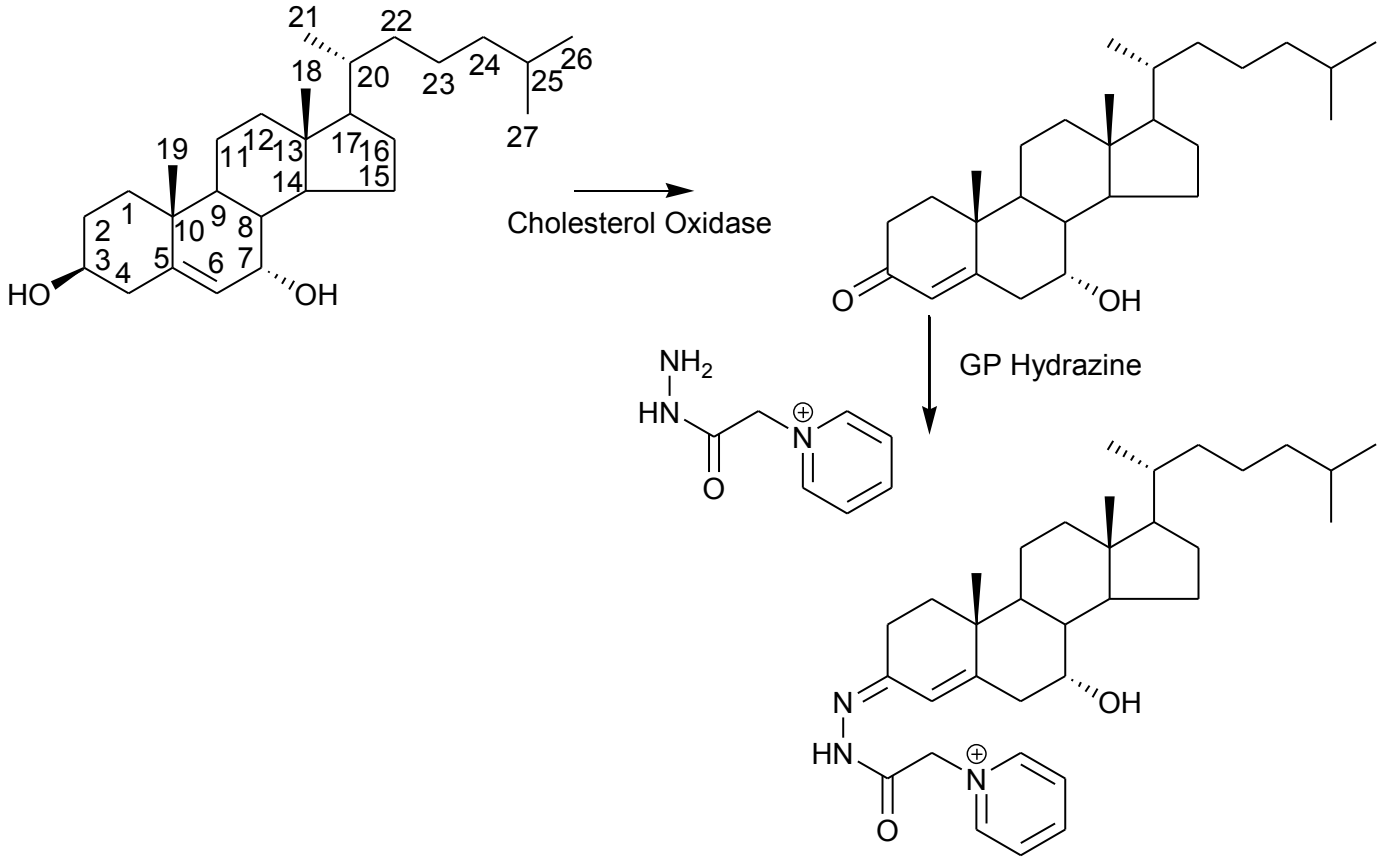

MS3: 534→455→
8.27 min
100
%RA
0
150
200
250
300
350
400
450
m/z
[M-107]+
427
[M-97]+
437
412
394
383
353
325
177
163
151
*b3-C2H4
*b1-12
*b2
'*f
'*e
-H
-H
OH
[M-79]+
N
HN
O
*b3-C2H4
*b1-12
*b2
‘*e
‘*f


MS3: 534→455→
10.53 min
[M-97]+
437
100
%RA
0
*c2+2H-H2O
[M-79]+
*b3-C2H4
N
HN
O
OH
*b1-12
*b2
[M-107]+
427
394
*b1-12
*b3-C2H4
*c2+2H-H2O
151
179
203
231
323
383
409
150
200
250
300
350
400
450
m/z
http://sterolanalysis.org.uk/



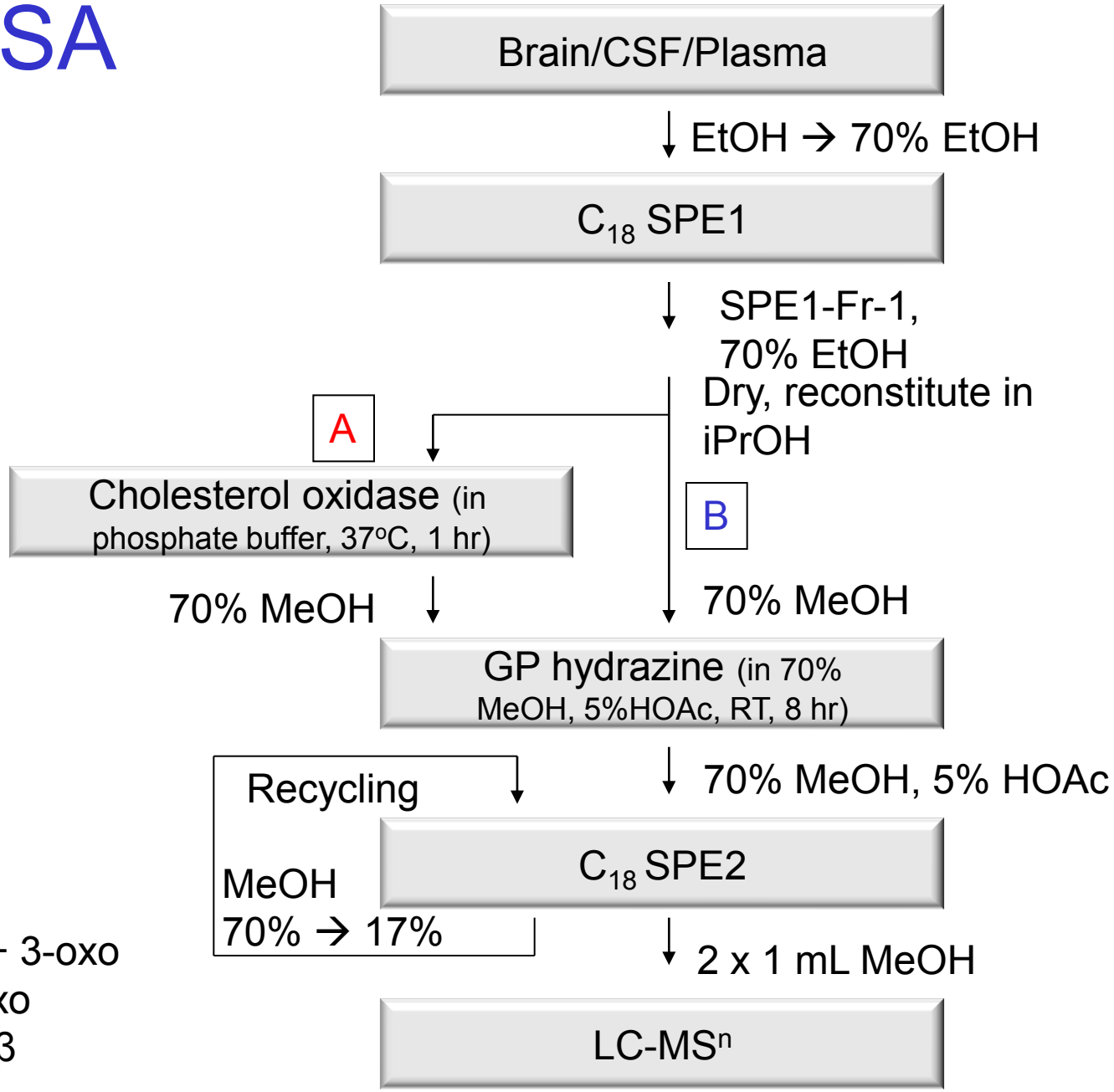
Griffiths WJ, et al. Free Radic Biol Med. 2012 Jul 27.

7α-Hydroxycholesterol & 7α-Hydroxycholest-4-en-3-one



Both present in plasma

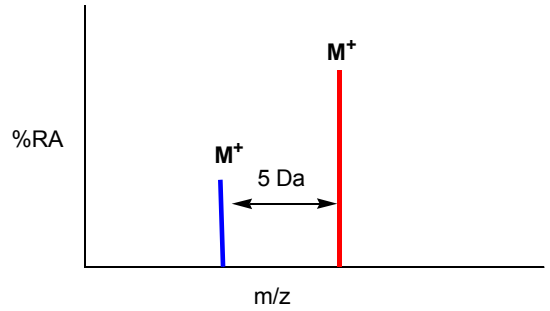
EADSA

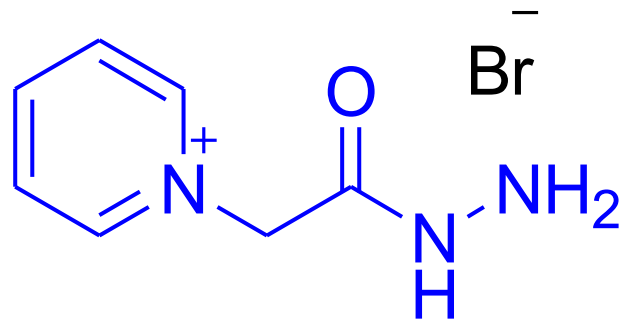


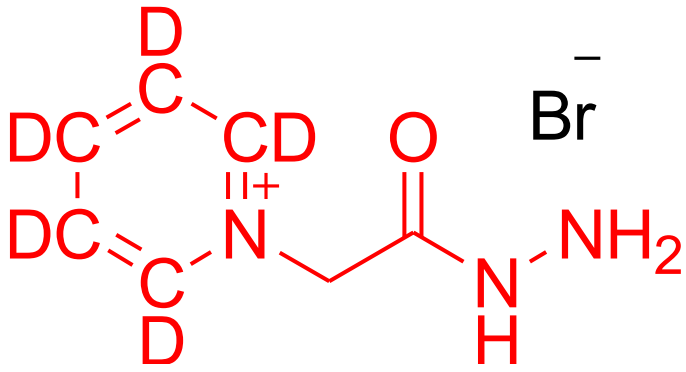
A = 3β + 3-oxo
B = 3-oxo
A-B = 3β

Stable Isotope Label

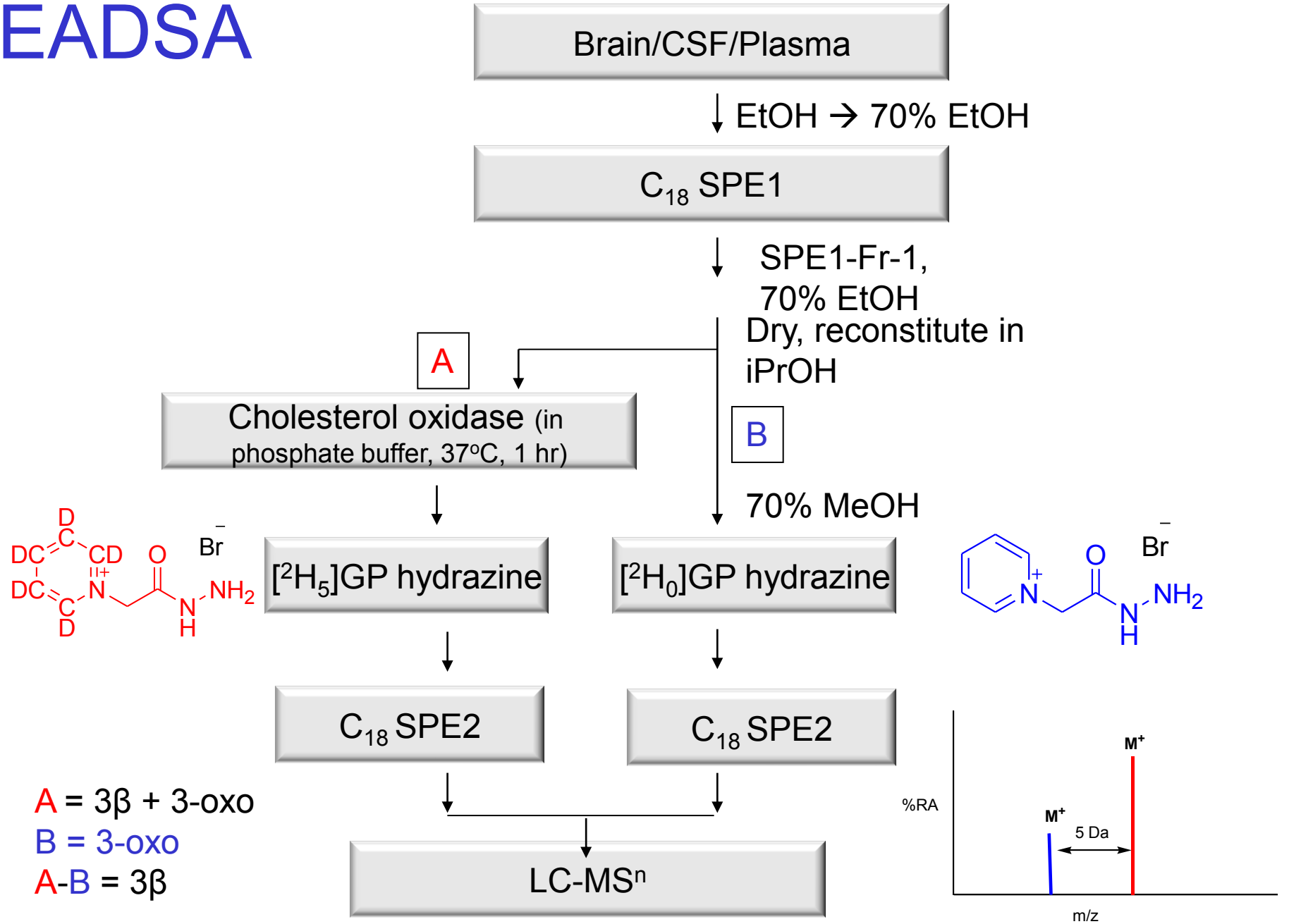
- Analyse A and B in a single LC-MS/MS run

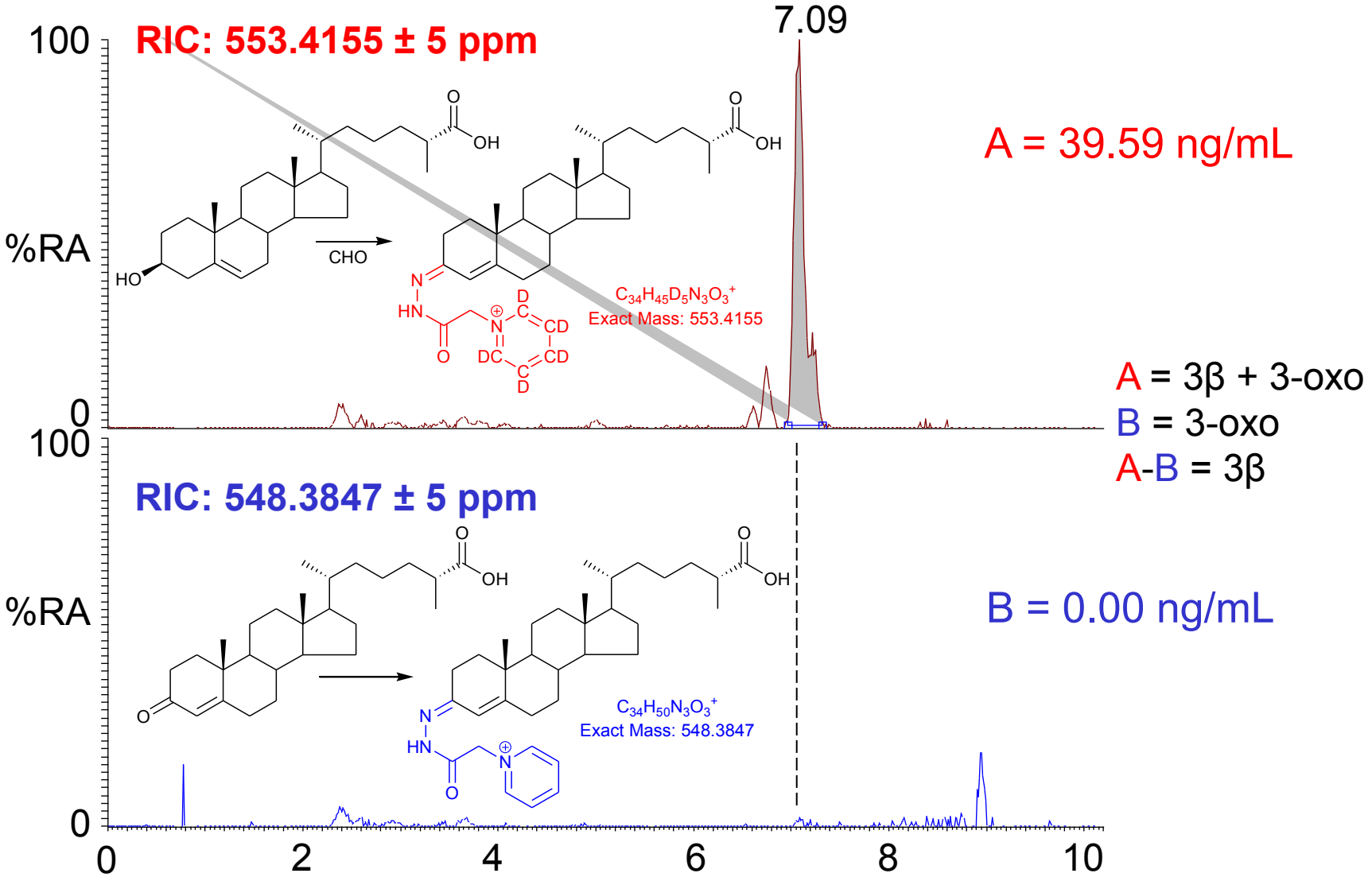


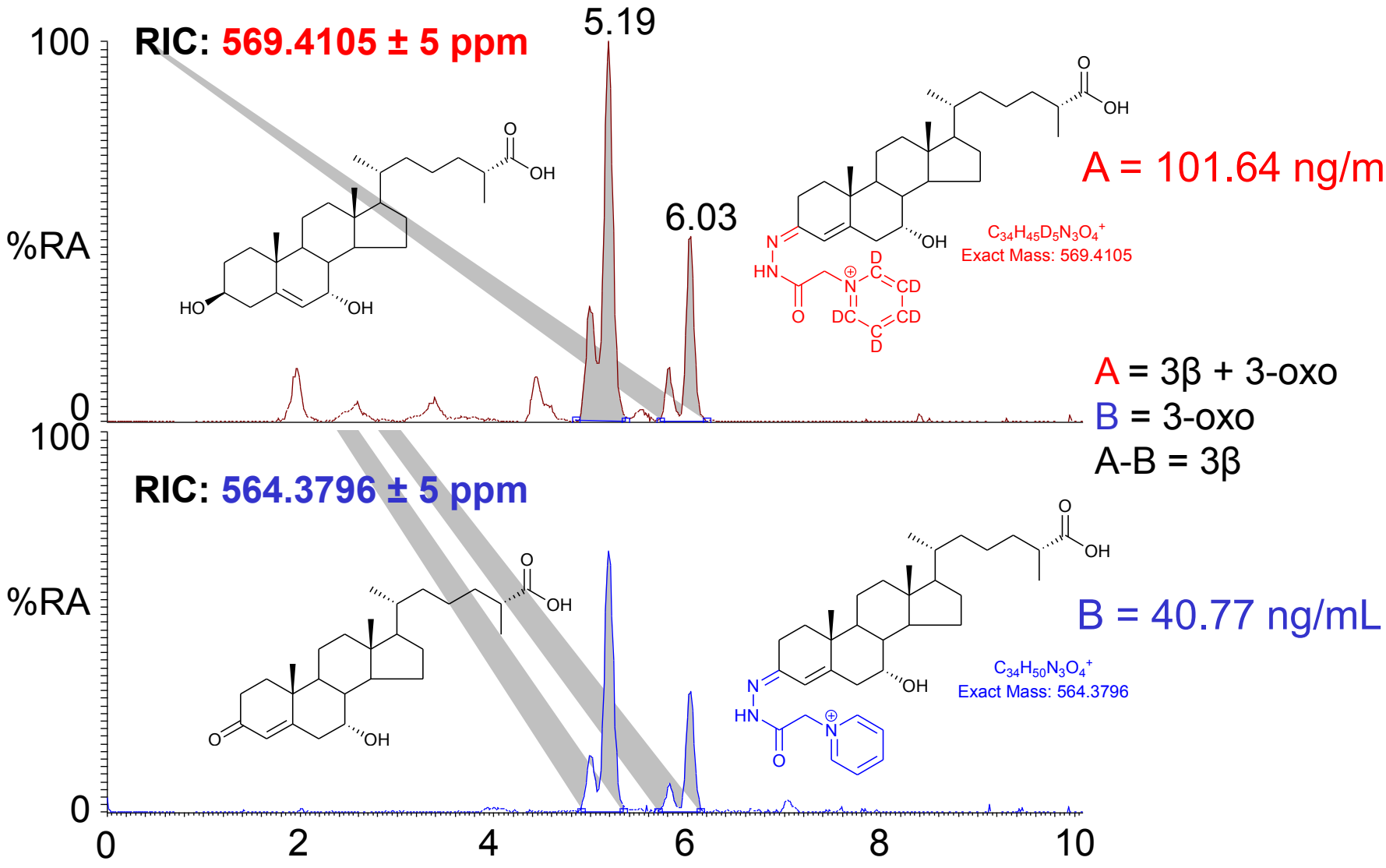


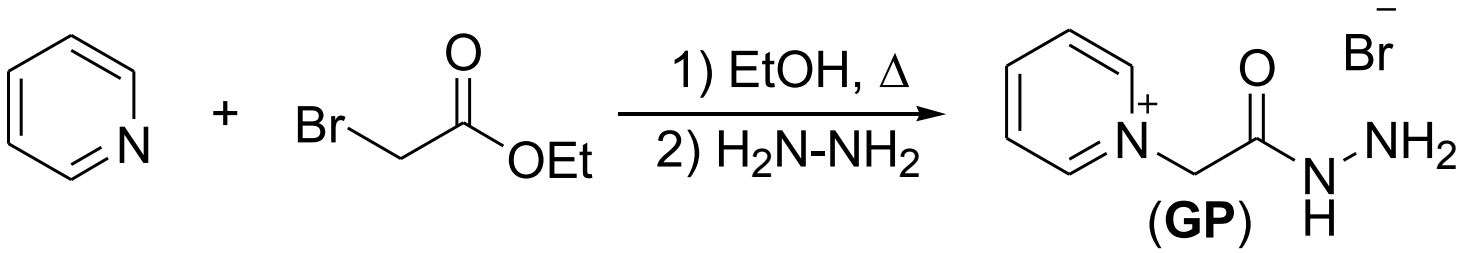


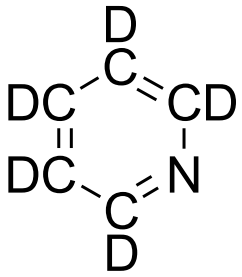
EADSA

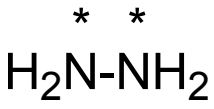

Brain/CSF/Plasma
EtOH → 70% EtOH
C_{18} SPE1
SPE1-Fr-1, 70% EtOH
Dry, reconstitute in iPrOH
A
B
Cholesterol oxidase (in phosphate buffer, 37°C, 1 hr)
70% MeOH
[$^2H_5$]GP hydrazine
[$^2H_0$]GP hydrazine
C_{18} SPE2
C_{18} SPE2
LC-MS^n
A = 3β + 3-oxo
B = 3-oxo
A-B = 3β
%RA
M+
M+
5 Da
m/z

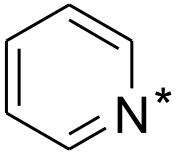

RIC: 553.4155 ± 5 ppm
7.09
100
%RA
0
HO
CHO
OH
O
N
HN
D
C
CD
DC
$C_{34}H_{45}D_5N_3O_3^+$
Exact Mass: 553.4155
A = 39.59 ng/mL
A = 3β + 3-oxo
B = 3-oxo
A-B = 3β
RIC: 548.3847 ± 5 ppm
$C_{34}H_{50}N_3O_3^+$
Exact Mass: 548.3847
B = 0.00 ng/mL
0
2
4
6
8
10

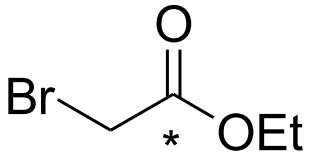

RIC: 569.4105 ± 5 ppm
5.19
6.03
100
%RA
0
HO
OH
O
OH
N
HN
O
D
C
CD
N
DC
CD
C
D
C34H45D5N3O4+
Exact Mass: 569.4105
A = 101.64 ng/mL
A = 3β + 3-oxo
B = 3-oxo
A-B = 3β
RIC: 564.3796 ± 5 ppm
100
%RA
0
O
OH
C34H50N3O4+
Exact Mass: 564.3796
B = 40.77 ng/mL
0
2
4
6
8
10
A-B = 60.87 ng/mL

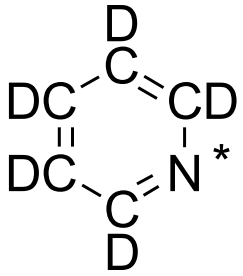

1) EtOH, Δ
2) H2N-NH2
(GP)

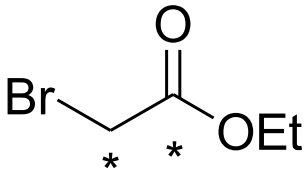


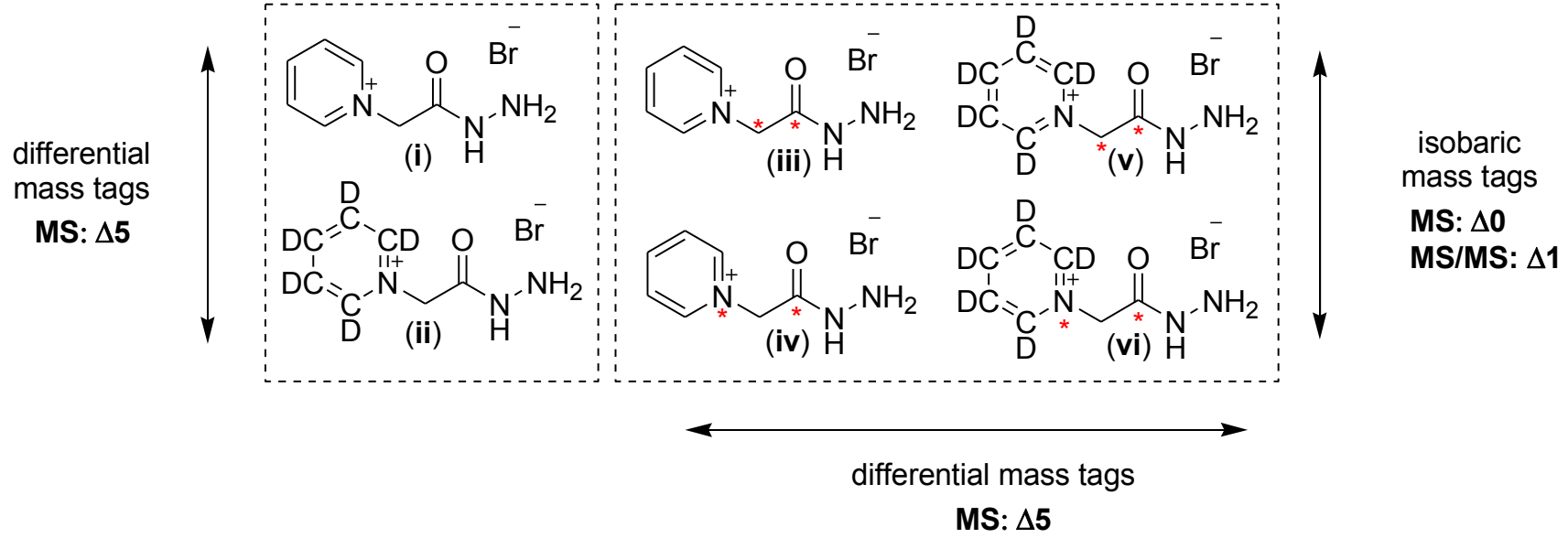

H2N-NH2

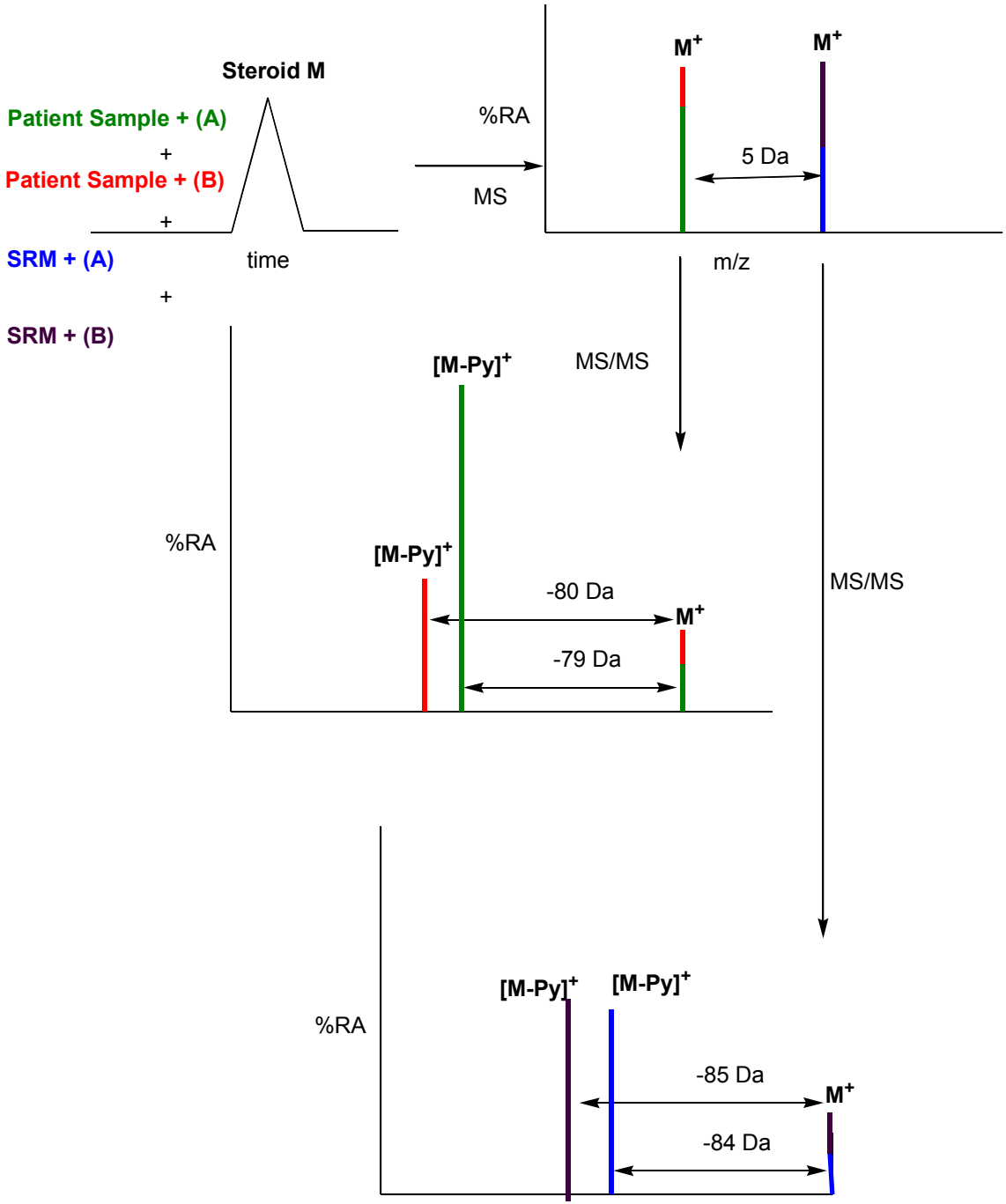


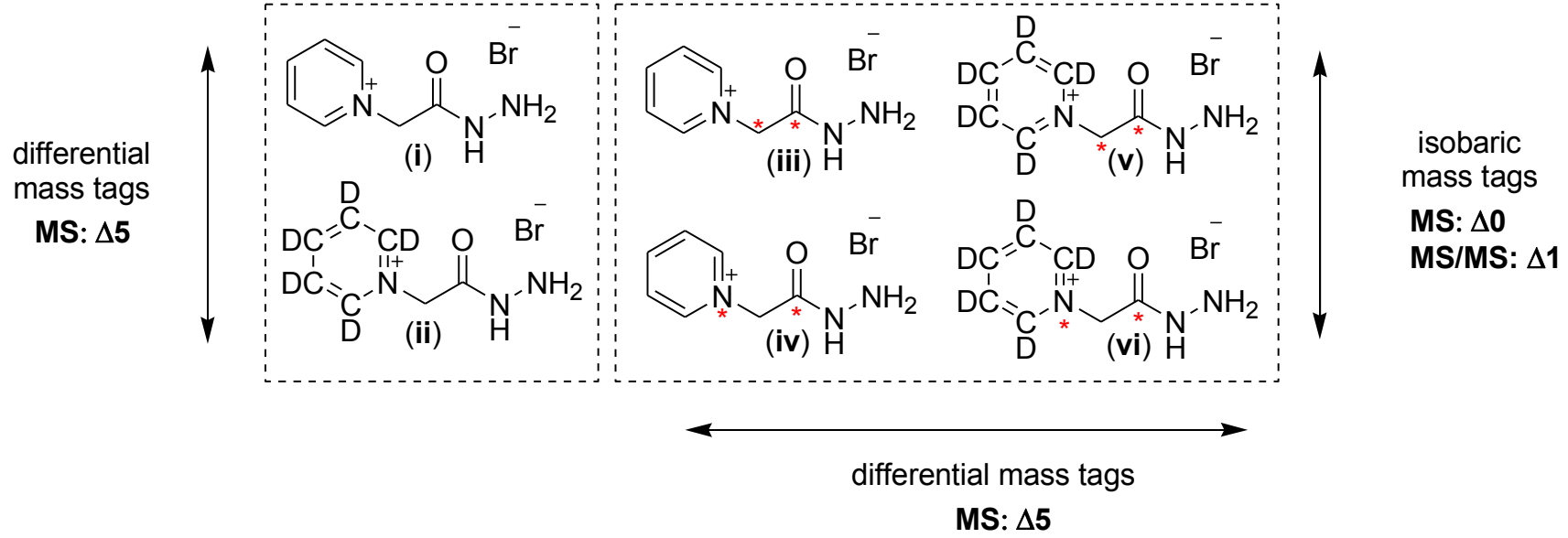


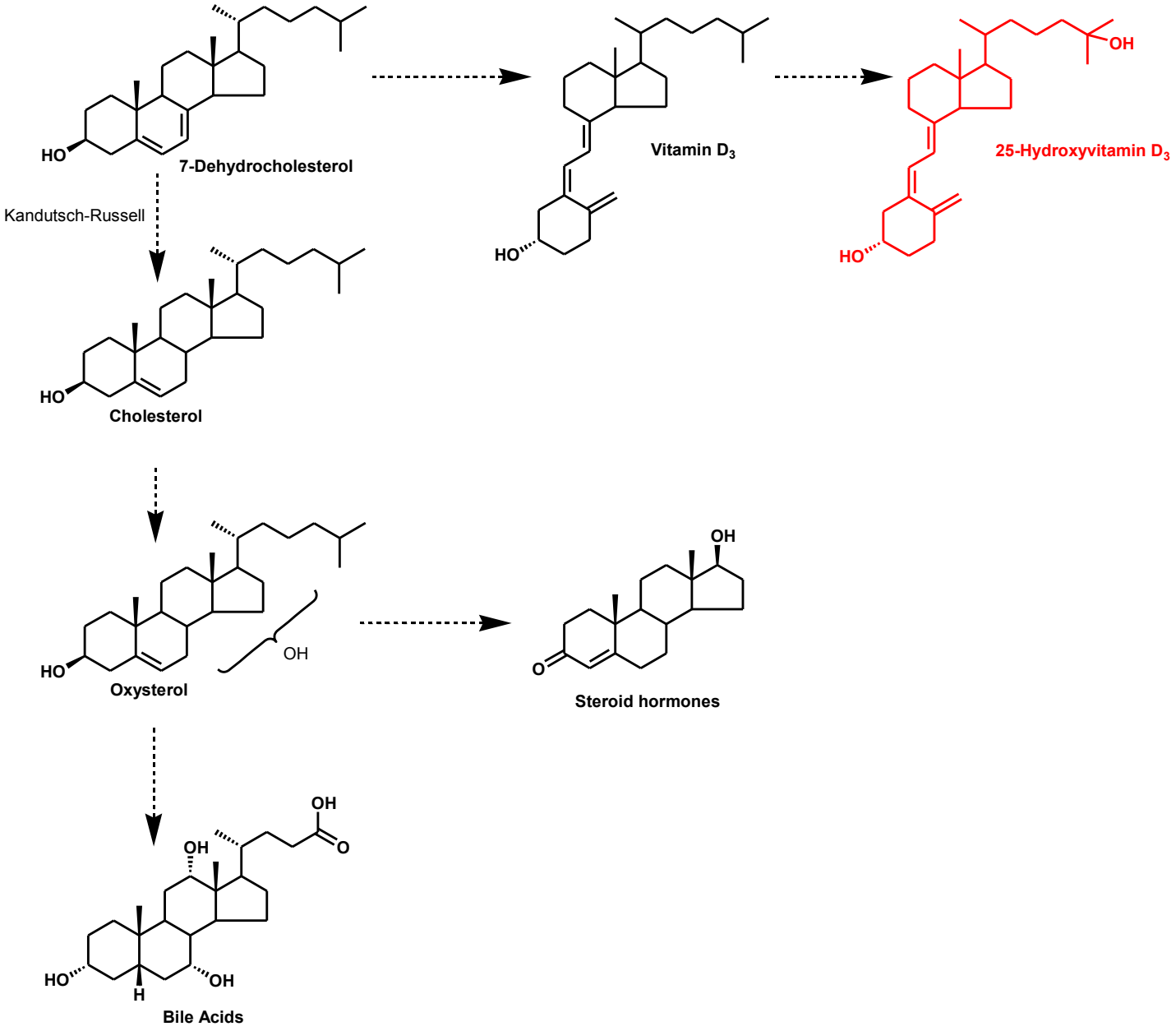


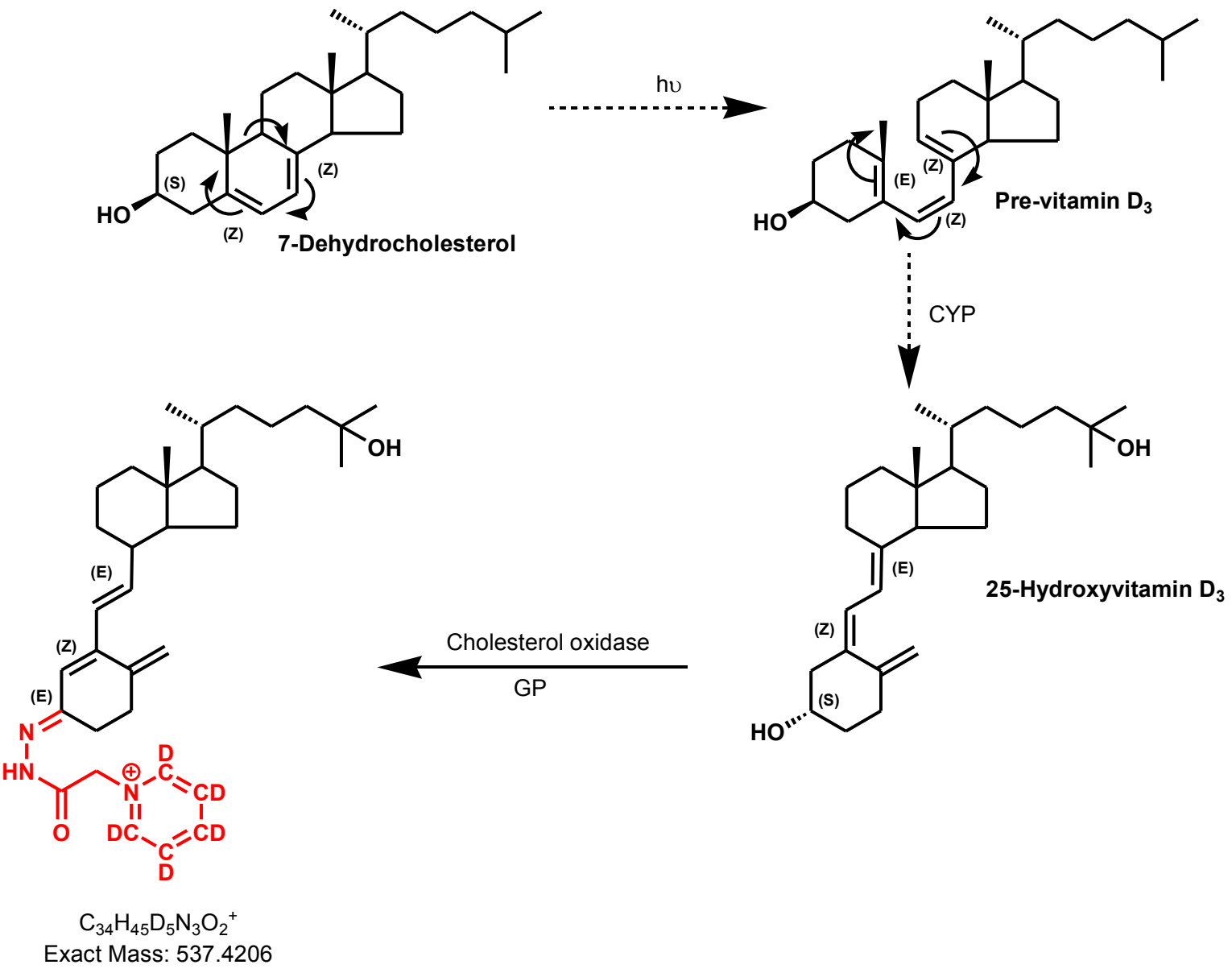



differential
mass tags
MS: Δ5
(i)
(ii)
(iii)
(iv)
(v)
(vi)
isobaric
mass tags
MS: Δ0
MS/MS: Δ1
differential mass tags
MS: Δ5

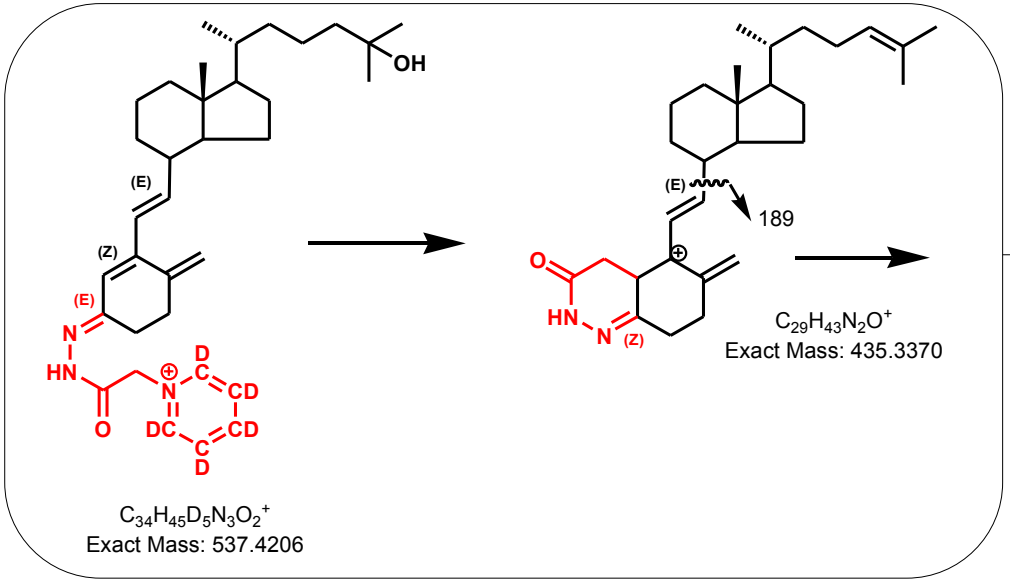

Steroid M
Patient Sample + (A)
+
Patient Sample + (B)
+
SRM + (A)
+
SRM + (B)
time
%RA
MS
M+
M+
5 Da
m/z
MS/MS
[M-Py]+
%RA
[M-Py]+
-80 Da
M+
-79 Da
MS/MS
%RA
[M-Py]+
[M-Py]+
-85 Da
M+
-84 Da


differential
mass tags
MS: Δ5
(i)
(ii)
(iii)
(iv)
(v)
(vi)
isobaric
mass tags
MS: Δ0
MS/MS: Δ1
differential mass tags
MS: Δ5


HO
7-Dehydrocholesterol
Kandutsch-Russell
HO
Cholesterol
HO
OH
Oxysterol
Vitamin D3
HO
OH
25-Hydroxyvitamin D3
HO
OH
O
Steroid hormones
OH
OH
O
HO
H
OH
Bile Acids


hυ
HO
(S)
(Z)
(Z)
7-Dehydrocholesterol
(E)
(Z)
(Z)
Pre-vitamin D3
CYP
OH
(E)
(Z)
(S)
HO
25-Hydroxyvitamin D3
Cholesterol oxidase
GP
OH
(E)
(Z)
(E)
N
HN
O
D
C
CD
N
DC
CD
C
D
C34H45D5N3O2+
Exact Mass: 537.4206

MS^3: 537→435→ $[M^+]$→$[M\text{-}Py\text{-}H_2O]^+$→



25-OHD_3: 4 – 12 ng/mL

Advantages & Disadvantages

- Enhance solubility (RP solvent)
- Enhance sensitivity (ESI)
- Specific MS^2 (-79) (GP-specific)
- Informative MS^3 (differentiate isomers)
- Quantitative

- Enzyme catalysis (purity, activity)
- Chemical derivatisation (remove excess)
- Side reactions (epoxide hydrolysis)
- *Syn/anti* conformers (dilute signal)
- Laborious

- Complementary to conventional LC-MS/MS and GC-MS

Y Wang
P. Crick
A. Meljon
I. Mathews
T. Bentley

Thermo Scientific
G. Woffendin
M. Hornshaw

Stockholm
J. Sjövall

UCL
P. Clayton

EPSRC
BBSRC

Swansea University
Prifysgol Abertawe

IP PRAGMATICS

Kit for quantitative detection of steroids

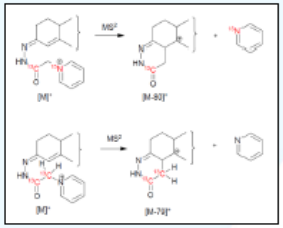
Researchers at Swansea University, Wales, have invented a novel technology for the quantitative detection of steroids using mass spectrometry. This vastly improved kit is more sensitive than existing methodologies and can detect and provide greater detail over a broad range of steroids.

Technology:

A steroid is an organic molecule containing a [illegible] of four joined cycloalkane rings. Examples include cholesterol, sex hormones estradiol and testosterone and anti-inflammatory drug dexamethasone. Steroids have been shown to play a major role respect to healthy aging, and abnormal cholesterol synthesis. Steroid metabolism has been implicated in numerous disease states (e.g. neurodegenerative disease; atherosclerosis; diabetes).

Despite growing evidence for structure-specific cholesterol metabolites having a wide range of regulatory and signalling properties, there are few methods for high-sensitivity structural determination and quantification in biological systems.

MS^2 fragmented oxosteroids derivatised with isotope-coded charge tags



Within the billion dollar market, global steroid analysis of biological samples is challenging leading to scarcity of quantitative standards. This is on account of extreme diversity of steroid natural products, tendency of a single steroid (or small steroid group) to dominate in abundance over all others, lack of a strong chromophore or readily ionised functional group,

Swansea have devised an integrated MS-based platform for quantitative and structural determination of cholesterol metabolites in biological systems; which involves incorporating quantitative charge tags with isobaric mass tags for quantifying steroids.

Applications

- Use within the NHS for monitoring patient response to treatment
- Measurement of vitamin D (25-OHD) levels
- Big pharma: measure effects of drug candidates
- Testing facility provided to pharma, hospitals, sports drug testing, commercial testing labs, etc.
- Investigations in related fields of systems biology, metabolomics, lipidomics, biomarker discovery, clinical screening and doping control.

Benefits

- Cholesterol metabolites can be quantified and structurally determined in biological systems
- Far more efficient, with potential for multiplex analyses that can greatly increase throughput
- Potentially identify "new" cholesterol metabolites

Patents:

Priority GB patent application filed in September 2012 and will follow onto PCT.

Status:

Swansea is seeking companies wishing to licence this technology or collaborate in co-development.

Licensing & Partnering Opportunity

For further information please contact:

Quinton Fivelman
e: quinton.fivelman@ip-pragmatics.com
t: +44 (0)20 3176 0580