SUPPLEMENTAL MATERIAL

Deep Mining of Oxysterols and Cholestenoic Acids in Human Plasma and Cerebrospinal Fluid: Quantification using Isotope Dilution Mass Spectrometry

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Supplemental Information

Supplemental Methods

Flowchart 1. Schematic illustration of sample preparation. Single-phase liquid extraction is performed into ethanol containing internal standards in the presence or absence of 0.35 M KOH.

Flowchart 2. Different protocols for single-phase liquid extraction.

Flowchart 3. Flowchart showing sterol identification and quantification routine.

Supplemental Figures and Tables

Figure S1. *Syn* (*Z*) and *anti* (*E*) conformers of the GP-derivative exemplified for 24S-HC. Also shown are 3D minimised energy structures (Chem 3D Pro). MS^2 fragmentation results in loss of the pyridine (Py) group with the formation of the $[M\text{-}Py]^+$ fragment ion.

Figure S2. LC-MS separation of GP-derivatised monohydroxycholesterols (HC). (A) Mass spectrometric peaks at *m/z* 546.48 corresponding to the $[M]^+$ ion of $[^2H_7]$24S-HC (upper panel); the $[M+1]^+$ ion of $[^2H_6]$26-HC (lower panel); and unresolved $[M]^+$ ion of $[^2H_7]$24R-HC and the $[M+1]^+$ ion of $[^2H_6]$26-HC (central panel). The structure of the $[M+1]^+$ ion of $[^2H_6]$26-HC is arbitrarily drawn with a ^{13}C at C-21. (B) Upper panel, TIC 534.4→455.4→ for monohydroxycholestenones in plasma. Lower panel TICs 541.5→462.4 for $[^2H_7]$-labelled monohydroxycholesterols.

Figure S3. MS^3 fragmentation of GP-derivatised monhydroxycholesterols in plasma. MS^3 ($[M]^+$→$[M\text{-}Py]^+$→) spectra of (A) 24S-HC; (B) 24R-HC; (C) $[^2H_7]$24S-HC; (D) $[^2H_7]$24R-HC; (E)25-HC; (F) $[^2H_6]$25-HC; (G) (25R)26-HC; (H) $[^2H_6]$(25R)26-HC; (I) $[^2H_7]$22R-HC; (J) 22S-HC; (K) $[^2H_7]$22S-HC. The formation of key fragment ions is illustrated in Figure S4A-D.

Figure S4. Key fragmentation routes in the MS^n spectra of GP-derivatised oxysterols. Mechanisms of side-chain cleavage are indicated by purple arrows. (A) 24R/S-HC and $[^2H_6]$24R/S-HC; (B) 25-HC and $[^2H_6]$25-HC; (C) (25R)26-HC and $[^2H_6]$(25R)26-HC; (D) 22R/S-HC and $[^2H_7]$22R/S-HC; (E) 7-HC and $[^2H_7]$7-HC; (F) 6β-HC and $[^2H_7]$6β-HC; (G) 7-OC and $[^2H_7]$7-OC; (H) 12α-HC; (I) 7α,25-diHC and $[^2H_6]$7α,25-diHC; (J) 7α,(25R/S)26-diHC and $[^2H_6]$7α,(25R/S)26-diHC; (K) 7α,12α-diHC; (L) 3β,7-diHCA(25R/S) and $[^2H_3]$3β,7-diHCA(25R/S); (M) 25-D_3 and $[^2H_6]$25-D_3; (N) 3βH-Δ^5-BA and 3β,7-diH-Δ^5-BA; (O) 3β-HCA; (P) 3β,7α,24-triHCA; (Q) 3β,7α,25-triHCA; (R) 3β,7α,12α-triHCA; (S) 3β,25-diHCA; (T) 3β,27-diHCA; (U) 3β,22,25-triHC-24O; (V) 3β,22-diHC-24O.

Figure S5. MS^3 fragmentation of GP-derivatised monohydroxycholesterols and monohydroxycholestenones in plasma. MS^3 ($[M]^+$→$[M\text{-}Py]^+$→) spectra of (A) 7β-HC; (B) $[^2H_7]$7β-HC; (C) 7α-HC; (D) $[^2H_7]$7α-HC; (E) 6β-HC (5α,6β-diHC-18), generated from the acid catalysed dehydration of 5α,6β-diHC; (F) $[^2H_7]$6β-HC ($[^2H_7]$5α,6β-18) generated from the acid catalysed dehydration of $[^2H_7]$5α,6β-diHC; (G) 7-OC; (H) $[^2H_7]$7-OC; (I) 7α-HCO; (J) $[^2H_7]$7α-HCO; (K) 12α-HC (no authentic standard). The formation of key fragment ions is illustrated in Figure S4E-H.

Figure S6. LC-MS separation of GP-derivatised dihydroxycholesterols, dihydroxycholestenones (diHCO), dihydroxycholestenoic (diHCA) and hydroxyoxocholestenoic (H,O-CA) acids. (A) RIC for the $[M]^+$ ions of (upper panel) 7α,25-diHC + 7α,25-diHCO and 7α,(25R/S)26-diHC + 7α,(25R/S)26-diHCO (555.4317 ± 5 ppm) found in plasma and (lower panel) $[^2H_6]$7α,25-diHC and $[^2H_6]$7α,(25R/S)26-diHC (561.4694 ± 5 ppm) over a 37 min gradient. (B) RIC of the $[M]^+$ ions of dihydroxycholestenones (550.4003 ± 5 ppm) found in plasma. Upper panel 37 min gradient, lower panel 17 min gradient. (C) RIC for the $[M]^+$ ions of (upper panel) 7αH,3O-CA(25R/S) (564.3796 ± 5 ppm) found in plasma and (lower panel) $[^2H_3]$7αH,3O-CA(25R/S) (567.3984 ± 5 ppm) over a 37 min gradient. (D) RIC for the $[M]^+$

ions of (upper panel) 7αH,3O-CA(25R/S) + 3β,7α-diHCA(25R/S) (569.4110 ± 5 ppm) found in plasma and (lower panel) [$^2H_3$]7αH,3O-CA(25R/S) (572.4298 ± 5 ppm) over a 37 min gradient. Coloured dashed lines indicate the coincidence of oxysterols between chromatograms.

Figure S7. MS^3 fragmentation of GP-derivatised dihydroxycholesterols (diHC) and dihydroxycholestenones (diHCO) in plasma. MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (A) 7α,25-diHC + 7α,25-diHCO; (B) [$^2H_6$]7α,25-diHC; (C) 7α,(25S)26-diHC + 7α,(25S)26-diHCO; (D) [$^2H_6$]7α,(25S)26-diHC; (E) 7α,(25R)26-diHC + 7αH,(25R)26-diHCO; (F) [$^2H_6$]7α,(25R)26-diHC; (G) 7α,25-diHCO; (H) 7α,(25R)26-diHCO; (I) 7α,12α-diHC. The formation of key fragment ions is illustrated in Figure S4I – S4K.

Figure S8. MS^3 fragmentation of GP-derivatised dihydroxycholestenoic (diHCA) and hydroxyoxocholestenoic (H,O-CA) acids in plasma. MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (A) 3β,7β-diHCA(25R); (B) 3β,7α-diHCA(25S) + 7αH,3O-CA(25S); (C) [$^2H_3$]3β,7α-diHCA(25S); (D) 3β,7α-diHCA(25R) + 7αH,3O-CA(25R); (E) [$^2H_3$]3β,7α-diHCA(25R); (F) 7αH,3O-CA(25S); (G) 7αH,3O-CA(25R); (H) [$^2H_3$]7αH,3O-CA(25R). The formation of key fragment ions is illustrated in Figure S4L.

Figure S9. LC-MS(MS^3) of cholesterol and other lipophilic sterols in plasma. (A) RIC for the $[M]^+$ ions of cholesterol (upper panel, 523.4419 ± 5 ppm), [$^2H_7$]cholesterol (central panel, 530.4858 ± 5 ppm) and isomers of cholestadien-3β-ol (lower panel 521.4262 ± 5 ppm). MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (B) cholesterol, (C) [$^2H_7$]cholesterol, (D) desmosterol (24-DHC) and (E) 8(9)-dehydrocholesterol (8-DHC). The formation of key fragment ions is illustrated in (F – H).

Figure S10. LC-MS separation of GP-derivatised 25-hydroxyvitanin D_3 and sterol-acids in plasma. (A) RIC for the $[M]^+$ ions of 25-D_3 (537.4211 ± 5 ppm, upper panel) and [$^2H_6$]25-D_3 (543.4588 ± 5 ppm, lower panel); (B) 3βH-Δ^5-BA (511.3691 ± 5 ppm); (C) 3β,7β-diH-Δ^5-BA and 3α,7α-diH-Δ^5-BA + 7αH,3O-Δ^4-BA (527.3640 ± 5 ppm, upper panel); 7αH,3O-Δ^4-BA (522.3326 ± 5 ppm, lower panel,); (D) 3β-HCA + 3O-CA (553.4161 ± 5 ppm, upper panel,); 3O-CA (548.3847 ± 5 ppm, lower panel); (E - H) 3β,7α,24-triHCA + 7α,24-diH,3O-CA, 3β,7α,25-triHCA + 7α,25-diH,3O-CA and 3β,7α,12α-triHCA + 7α,12α-diH,3O-CA (585.4059 ± 5 ppm, upper panels); (E) Lower panel, 7α,24-diH,3O-CA, 7α,25-diH,3O-CA and 7α,12α-diH,3O-CA (580.3739 ± 5 ppm); (F – H) Lower panels show MRM chromatograms targeting (F) 3β,7α,24-triHCA + 7α,24-diH,3O-CA; (G) 3β,7α,25-triHCA + 7α,25-diH,3O-CA; (H) 3β,7α,12α-triHCA and 7α,12α-diH,3O-CA. Authentic standards are not available for 3β,7α,12α-triHCA and 7α,12α-diH,3O-CA. Coloured dashed lines indicate the coincidence of oxysterols between chromatograms. MS^3 spectra are presented in Figures S11 & S12.

Figure S11. MS^3 fragmentation of GP-derivatised 25-hydroxyvitamin D_3 and sterol acids in plasma. MS^3 ($[M]^+ \rightarrow [M\text{-}Py\text{-}H_2O]^+ \rightarrow$) spectra of (A) 25-$D_3$ and (B) [$^2H_6$]25-D_3. MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (C) 3βH-$\Delta^5$-BA; (D) 3β,7β-diH-$\Delta^5$-BA; (E) 3β,7α-diH-$\Delta^5$-BA + 7αH,3O-$\Delta^4$-BA; (F) 7αH,3O-$\Delta^4$-BA; (G) 3β-HCA + 3O-CA; (H) 3O-CA. The formation of key fragment ions is illustrated in Figure S4M-O.

Figure S12. MS^3 fragmentation of GP-derivatised of trihydroxycholestenoic (triHCA) and dihydroxy-3-oxocholestenoic acids (diH,3O-CA) in plasma. MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (A) 3β,7α,24-triHCA + 7α,24-diH,3O-CA; (B) 3β,7α,25-triHCA + 7α,25-diH,3O-CA; (C) 3β,7α,12α-triHCA + 7α,12α-diH,3O-CA (no authentic standard). The formation of key fragment ions is illustrated in Figure S4P-R.

Figure S13. MS^3 fragmentation of GP-derivatised oxysterols in plasma. MS^3 spectra of the postulated structures (A) 3β,25-diHCA or *3β,25,x-trihydroxycholest-5-en-y-one* (no authentic standards available); (B) *3β,27-diHCA* or 3β,27,x-trihydroxycholest-5-en-y-one (no authentic standards available); (C) 3β,x-diHCA or 3β,x,y-trihydroxycholest-5-en-z-one (no authentic standards available); (D) *3β,22,25-trihydroxycholest-5-en-24-one* or 3β,x-diHCA (no authentic standard); (E) *3β,22-dihydroxycholest-5-en-24-one* or 3β,20-dihydroxycholest-5-en-22-one (no authentic standard); (F) 7α,x-diH,3O-CA (no

authentic standard). The more likely structures are given in *italics.* The formation of key fragment ions is illustrated in Figure S4S-V.

Figure S14. MS^3 fragmentation of GP-derivatised of trihydroxycholestenoic (triHCA) and dihydroxy-3-oxocholestenoic acids (diH,3O-CA) in CSF. MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (A) 3β,7α,24-triHCA + 7α,24-diH,3O-CA; (B) 3β,7α,25-triHCA + 7α,25-diH,3O-CA; (C) 3β,7α,12α-triHCA + 7α,12α-diH,3O-CA (no authentic standard) and (D) 3β,7α,x-triHCA + 7α,x-diH,3O-CA. The formation of key fragment ions is illustrated in Figure S4P-R.

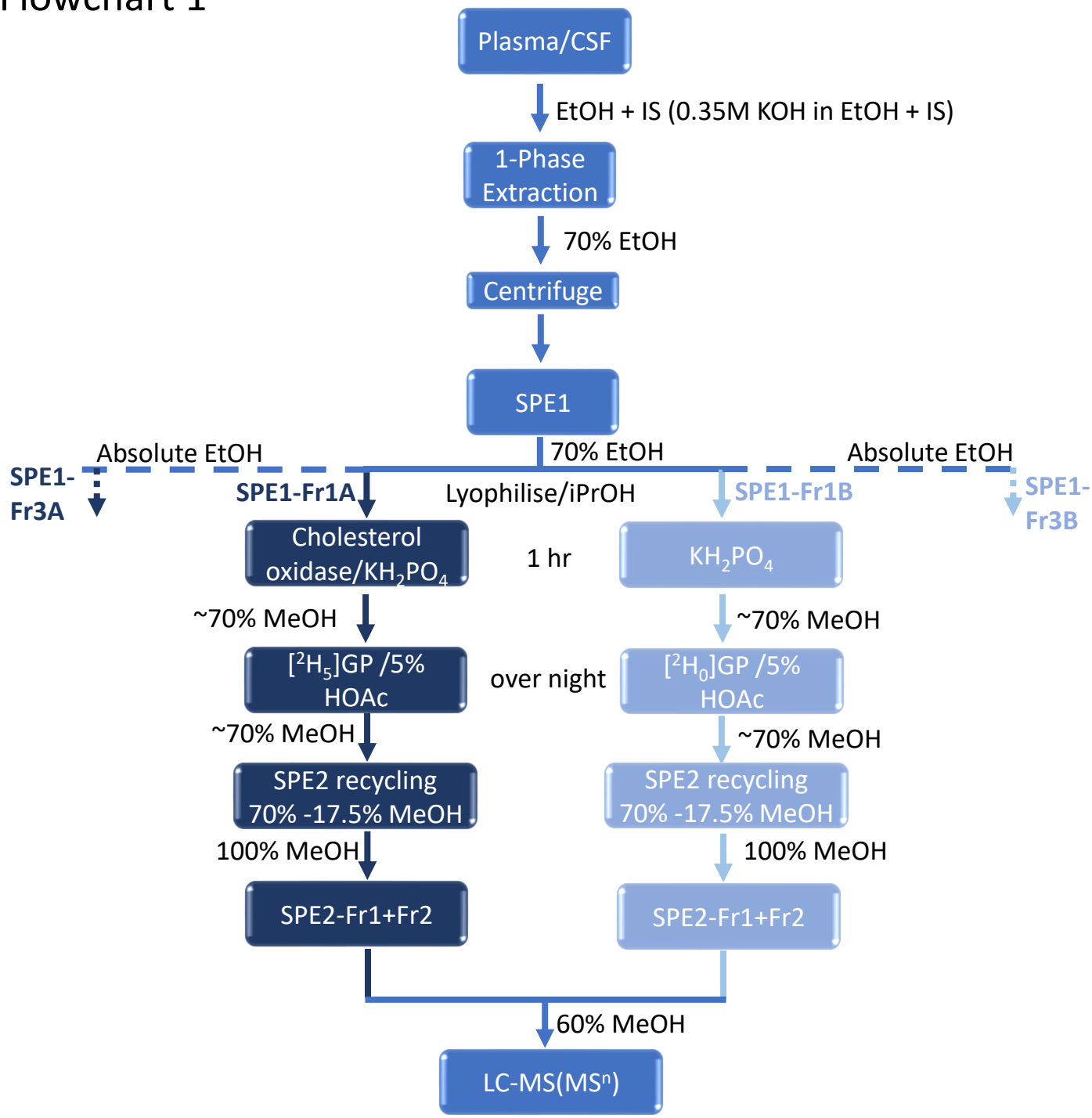
Table S1. Systematic and common names of sterols including oxysterols and sterol-acids identified or partially identified in the current study. Supplier of reference standards are indicated when available.

Table S2. Figures of merits when varying amount of OxysterolSPLASH at a plasma volume of 100 µL.

Table S3. Figures of merit revealed by standards additions to (A) 100 µL of plasma using 50 µL of OxysterolSPLASH and (B) 100 µL CSF using 20 µL of OxysterolSPLASH.

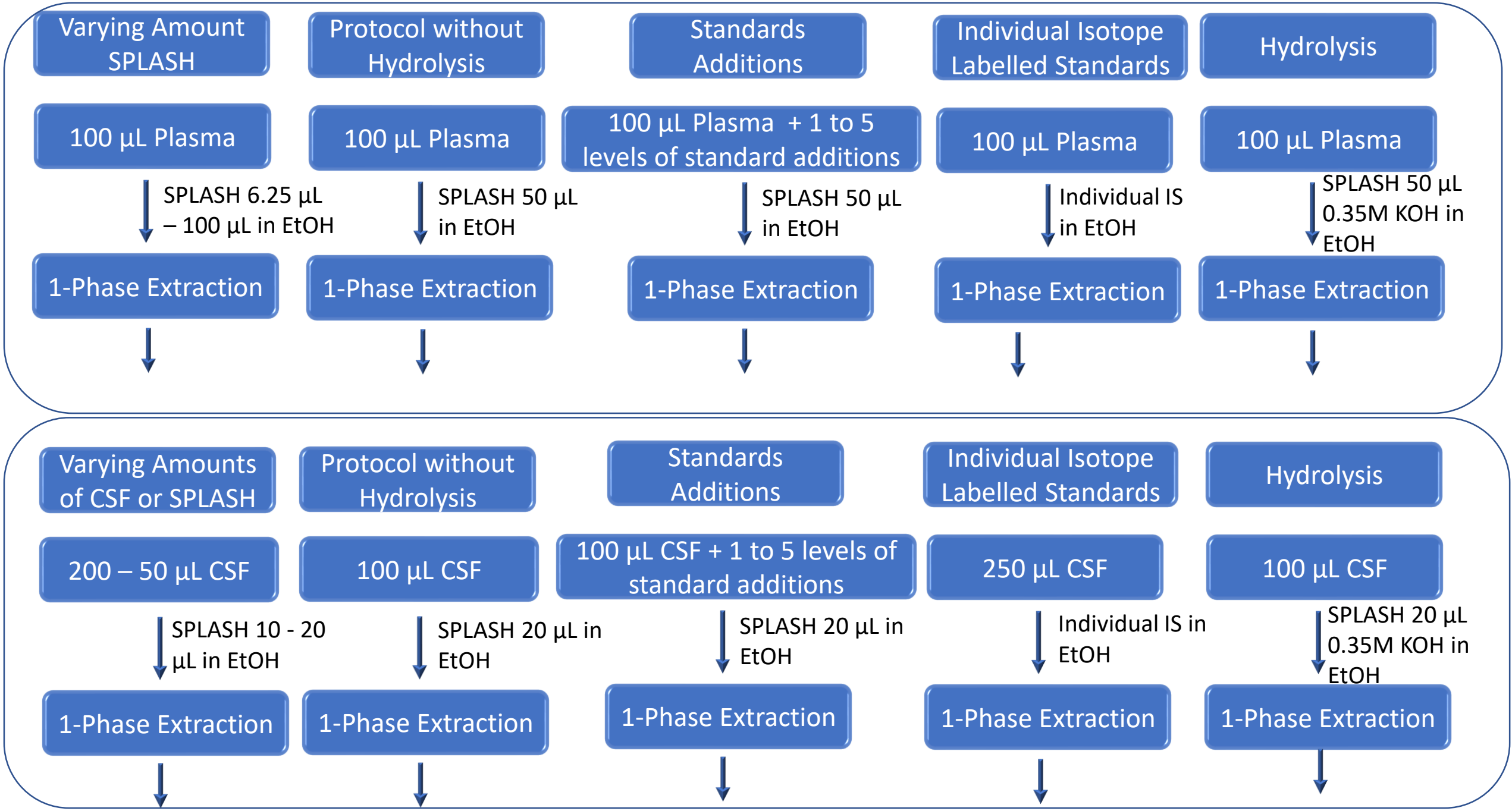
Table S4. Diagnostic fragment ions and simple rules for structure determination of GP-derivatised sterols.

Flowchart 1

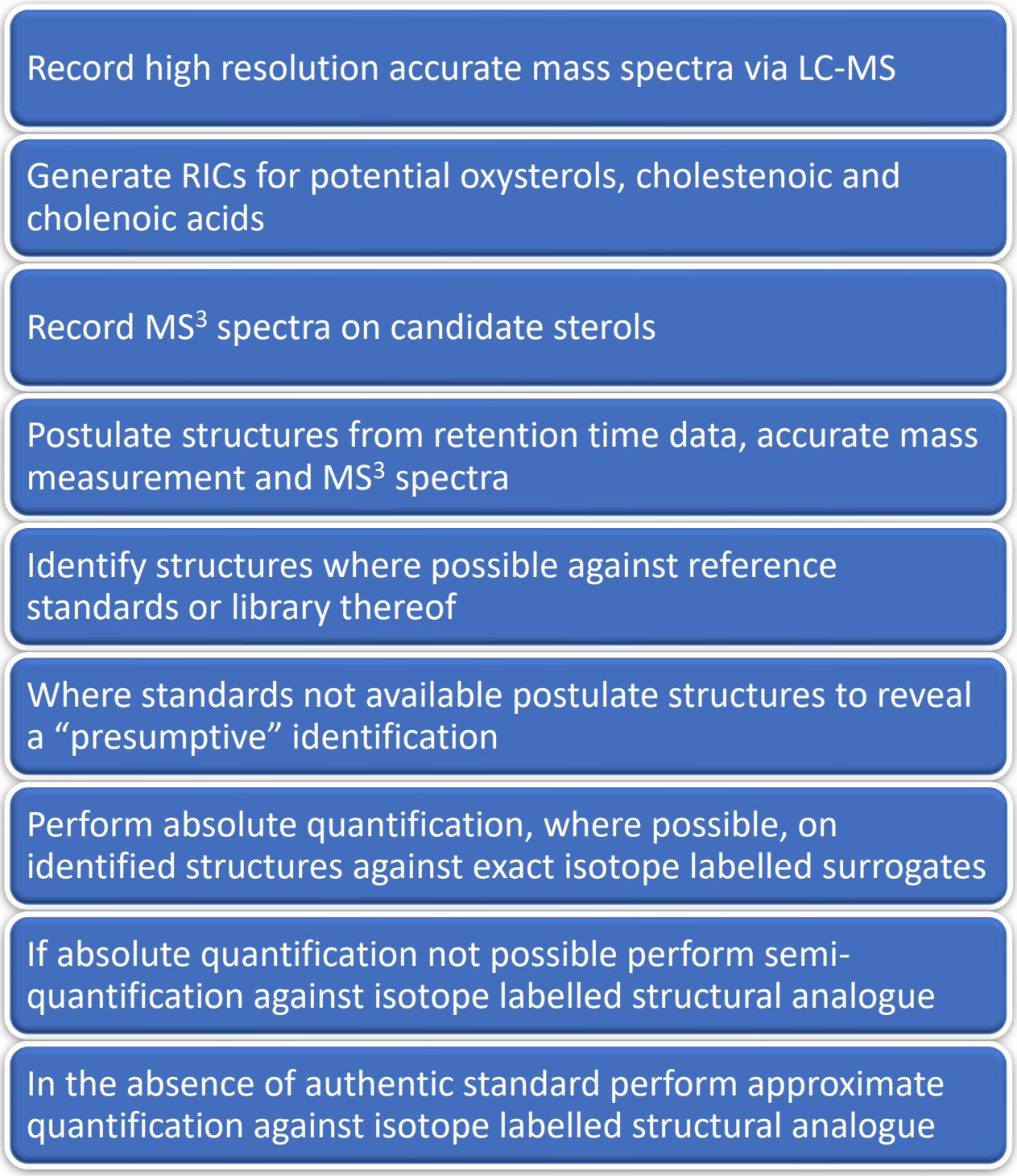


Schematic illustration of sample preparation. Single-phase liquid extraction is performed into ethanol containing internal standards in the presence or absence of 0.35 M KOH

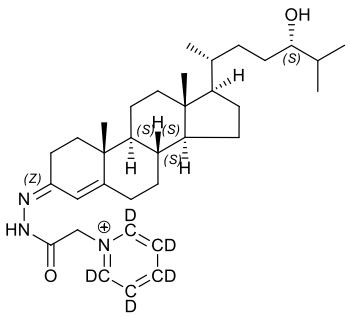
Flowchart 2. Different protocols for single-phase liquid extraction.



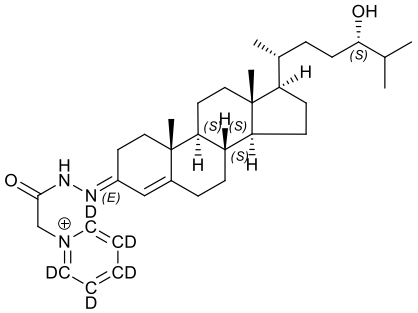
Flowchart 3



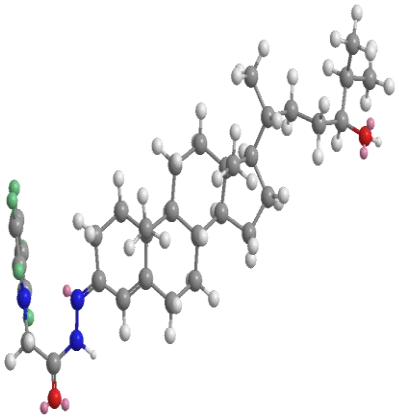
Flowchart showing sterol identification and quantification routine



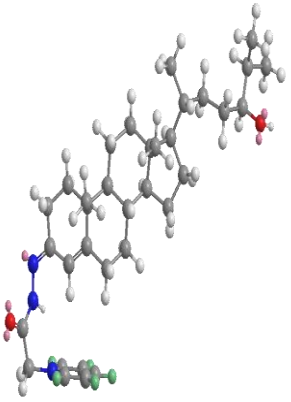
Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

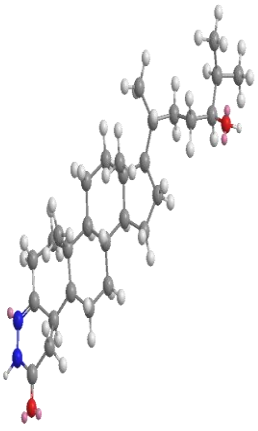


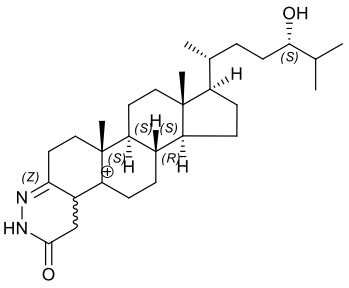
Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368



MS²







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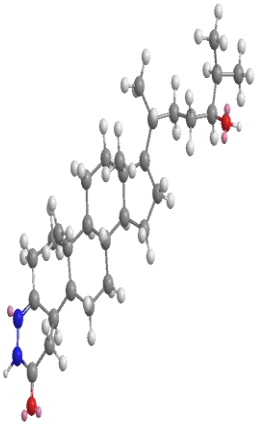
Exact Mass: 455.3632

$[M\text{-}Py]^+$



S2A

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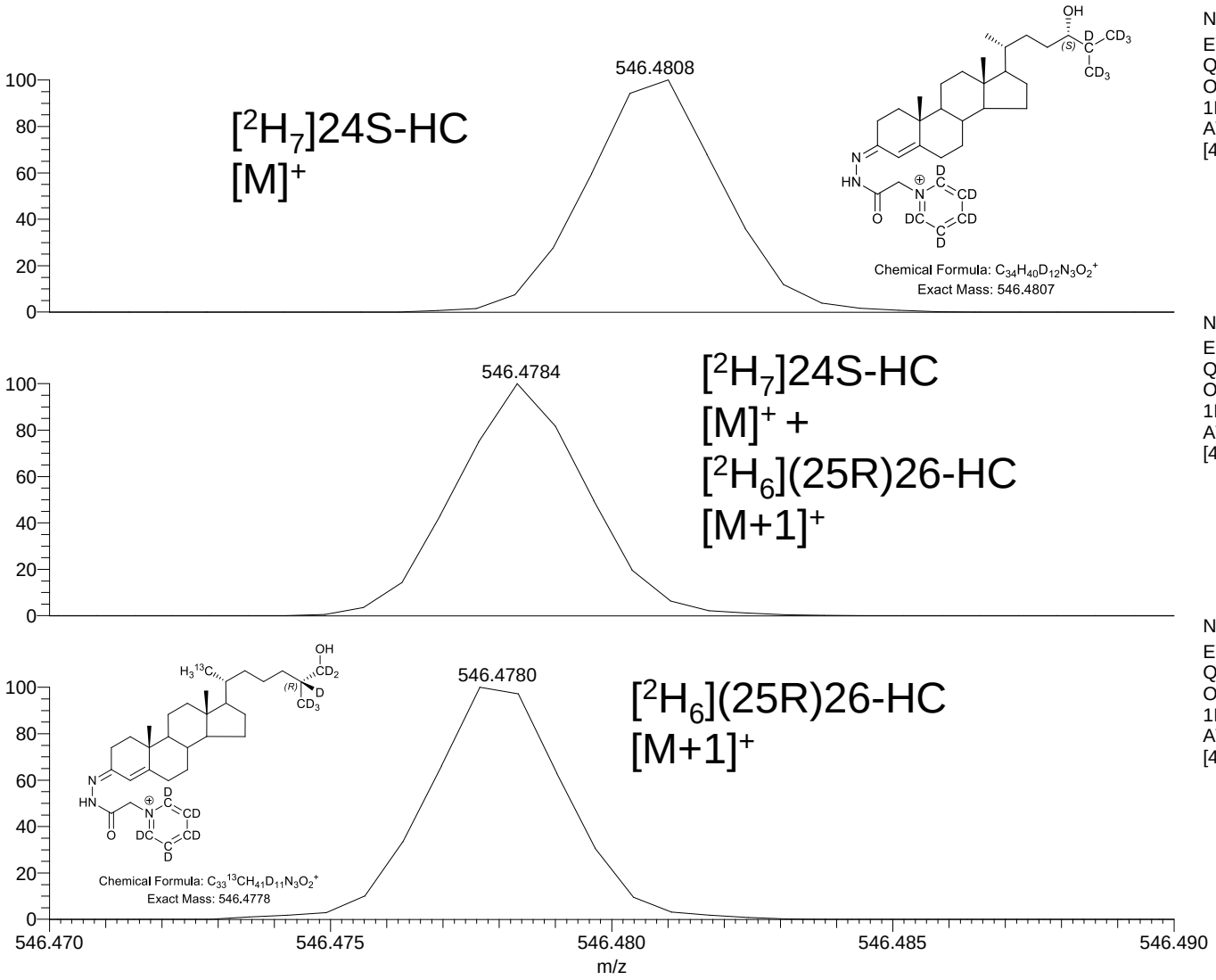

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NL: 2.16E5
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AV: 1 F: FTMS + p ESI Full ms [400.00-610.00]

NL: 1.77E5
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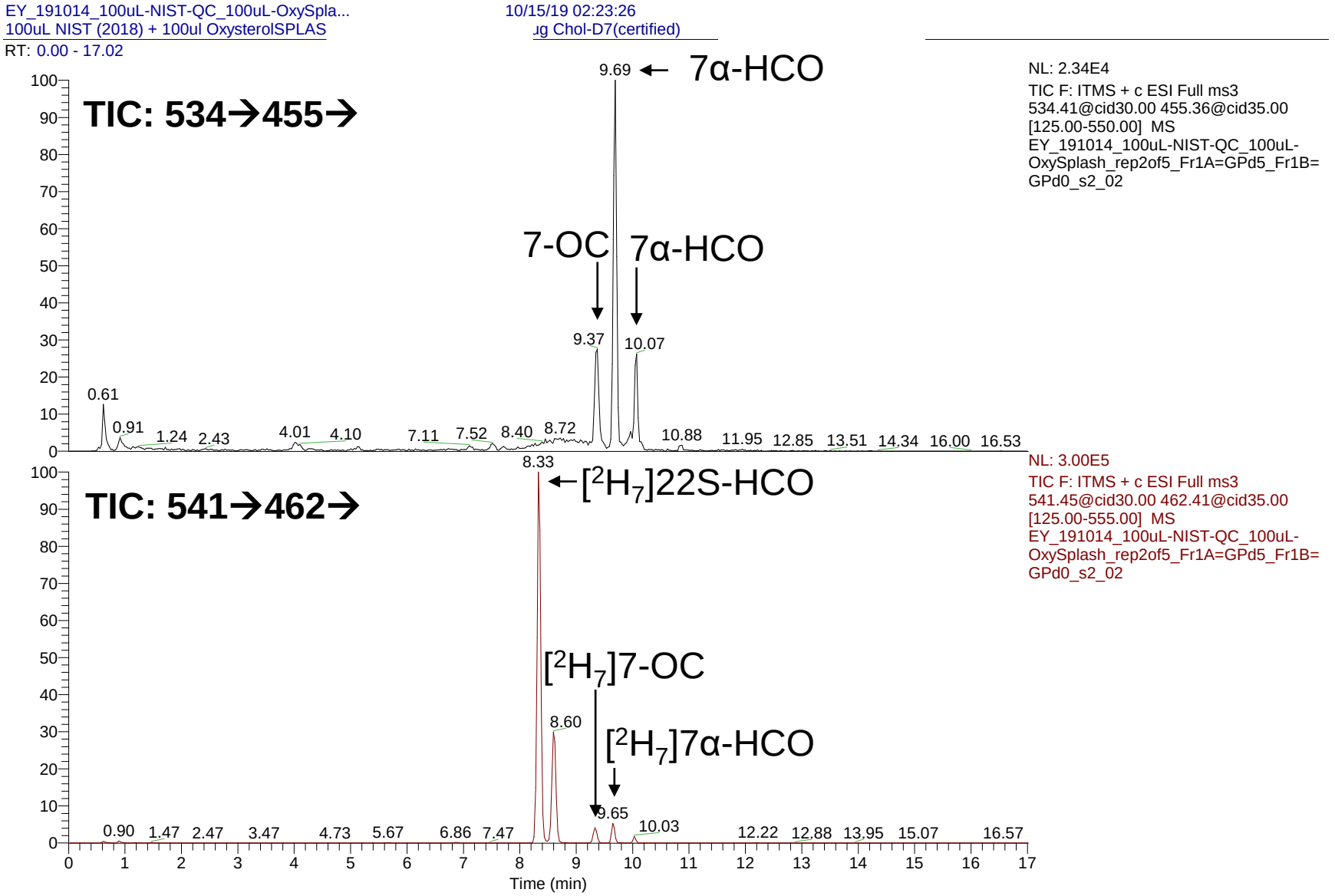
S2B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7-OC
7α-HCO
9.37
10.07
0.61
0.91
1.24
2.43
4.01
4.10
7.11
7.52
8.40
8.72
10.88
11.95
12.85
13.51
14.34
16.00
16.53
NL: 2.34E4
TIC F: ITMS + c ESI Full ms3
534.41@cid30.00 455.36@cid35.00
[125.00-550.00] MS
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GPd0_s2_02
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[2H7]7-OC
8.60
[2H7]7α-HCO
9.65
10.03
0.90
1.47
2.47
3.47
4.73
5.67
6.86
7.47
12.22
12.88
13.95
15.07
16.57
NL: 3.00E5
TIC F: ITMS + c ESI Full ms3
541.45@cid30.00 462.41@cid35.00
[125.00-555.00] MS
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GPd0_s2_02
Time (min)

S3A

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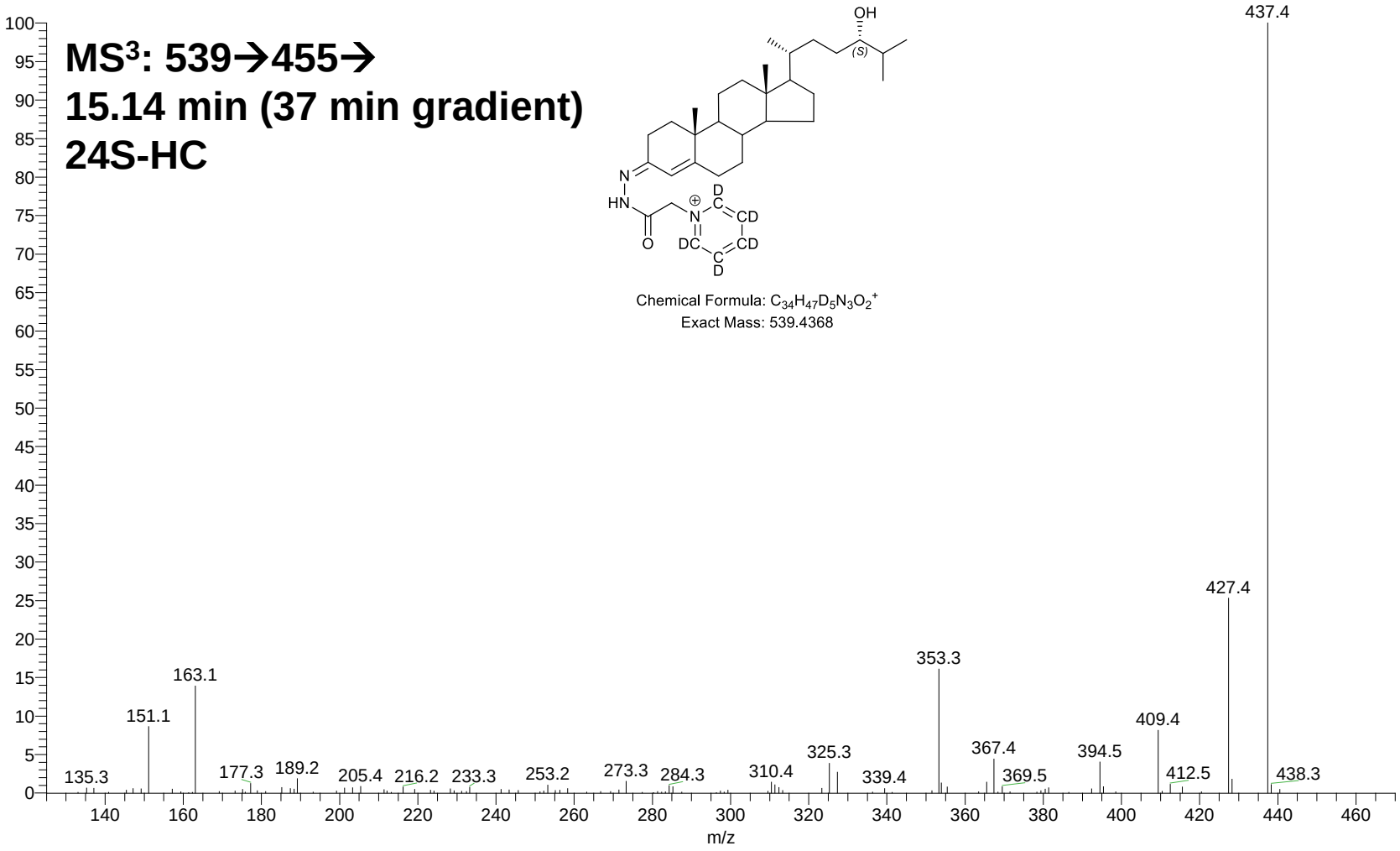

S3B

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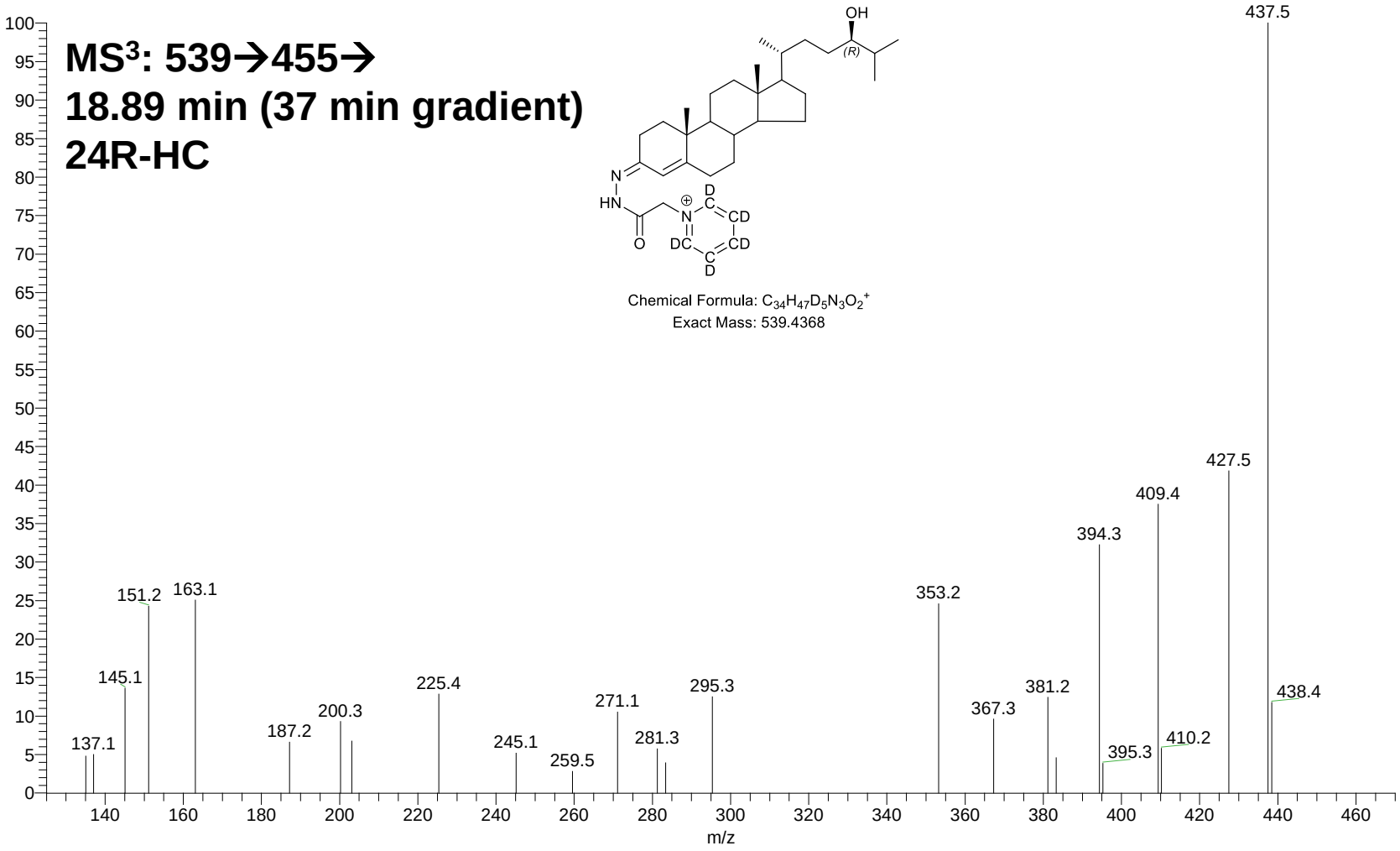

S3C

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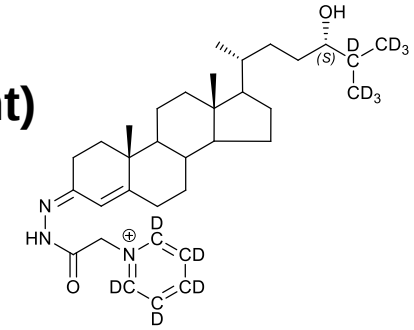
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

14.92 AV: 1 NL: 1.24E4

MS^3: 546→462→
14.92 min (37 min gradient)
$[^2H_7]$24S-HC



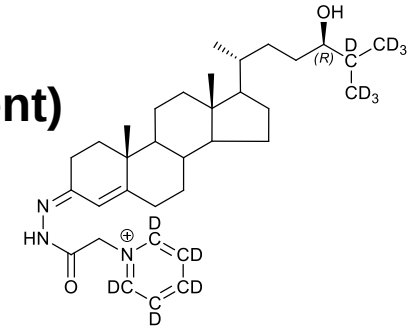
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

S3D

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

18.71 AV: 1 NL: 4.78E3

MS^3: 546→462→
18.71 min (37 min gradient)
$[^2H_7]$24R-HC



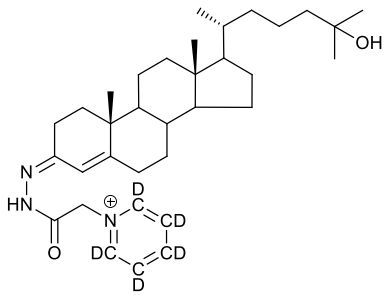
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

S3E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

15.94 AV: 1 NL: 2.08E3

MS^3: 539→455→
15.94 min (37 min gradient)
25-HC

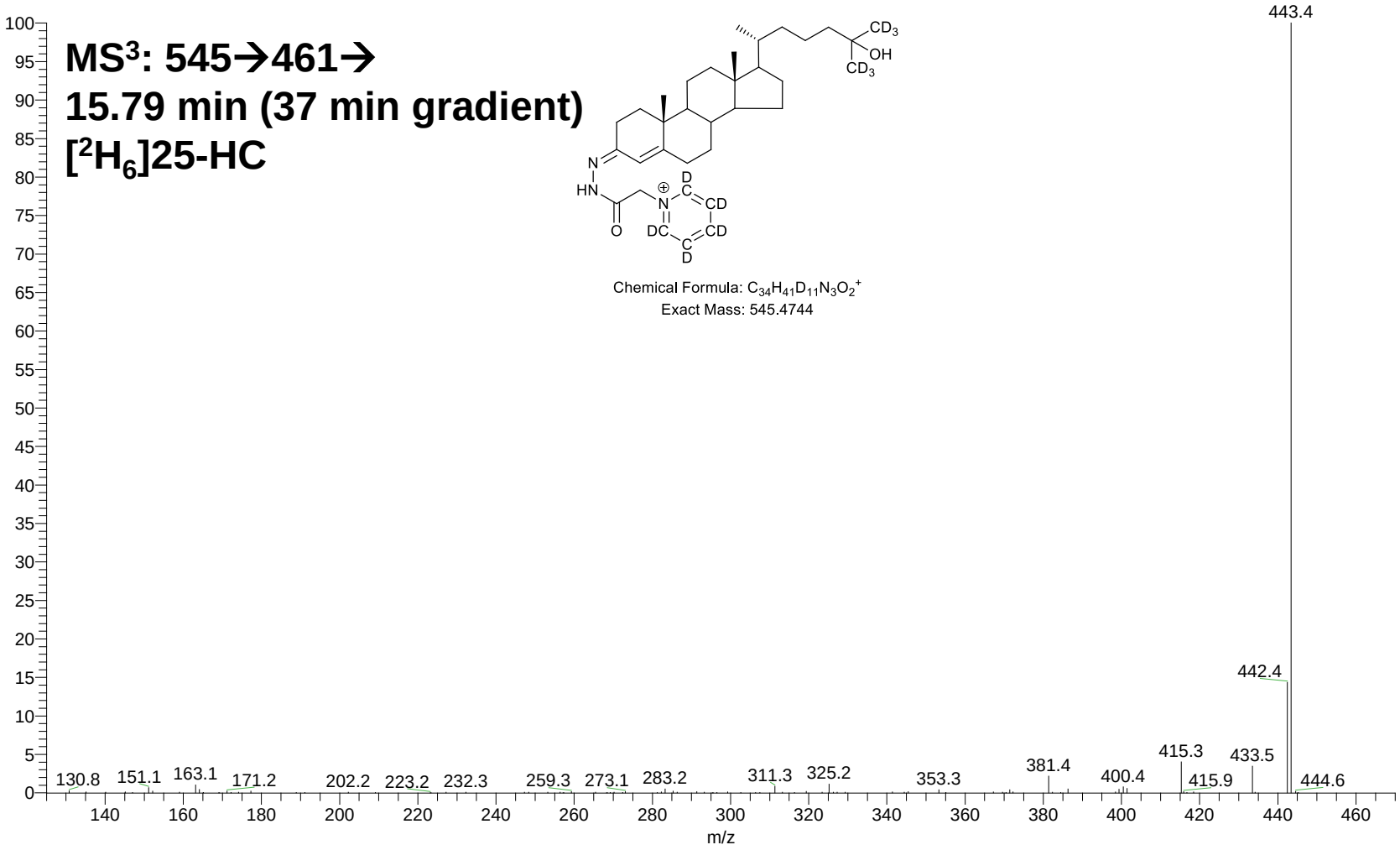


Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

S3F

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 545.47@cid30.00 461.40@cid35.00 [125.00-555.0

15.79 AV: 1 NL: 1.12E4

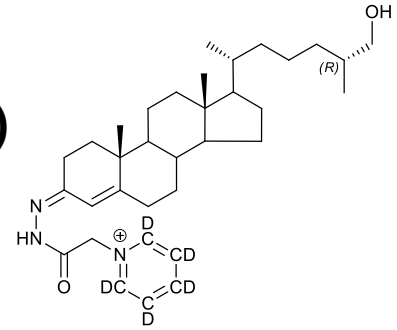


S3G

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

17.75 AV: 1 NL: 4.63E3

MS^3: 539→455→
17.75 min (37 min gradient)
(25R)26-HC

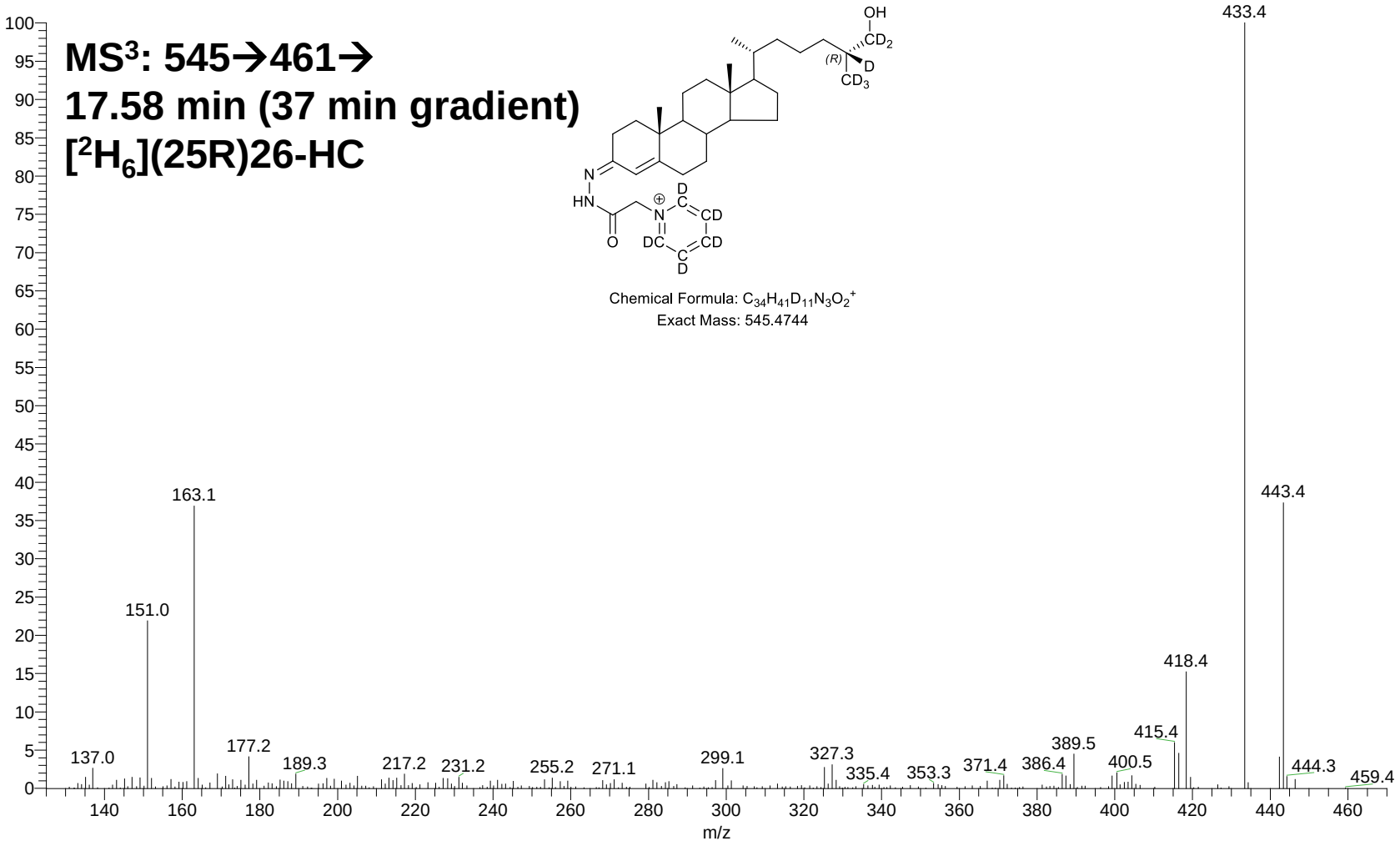


Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

S3H

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 545.47@cid30.00 461.40@cid35.00 [125.00-555.0

17.58 AV: 1 NL: 2.24E4

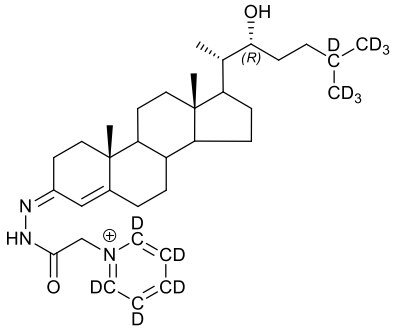


S3I

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

7.86 AV: 1 NL: 1.28E3

MS^3: 546→462→
7.86 min (37 min gradient)
[$^2H_7$]22R-HC



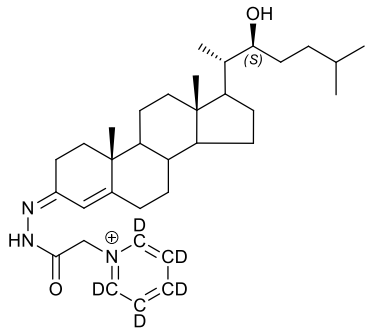
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

S3J

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

8.35 AV: 1 NL: 4.92E1

MS^3: 539→455→
8.35 min (17 min gradient)
22S-HC



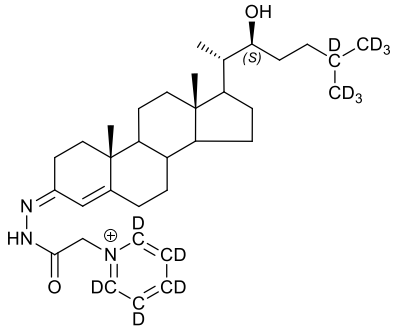
Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

S3K

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

8.34 AV: 1 NL: 1.04E5

MS^3: 546→462→
8.34 min (17 min gradient)
[$^2H_7$]22S-HC

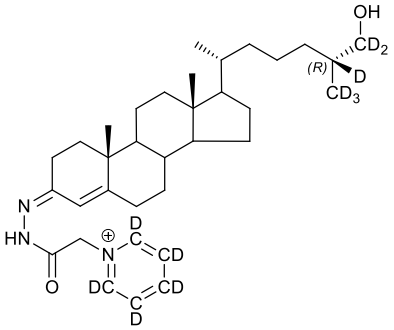


Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:44:12
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 545.47@cid30.00 461.40@cid35.00 [125.00-555.0

18.35 AV: 1 NL: 6.81E3

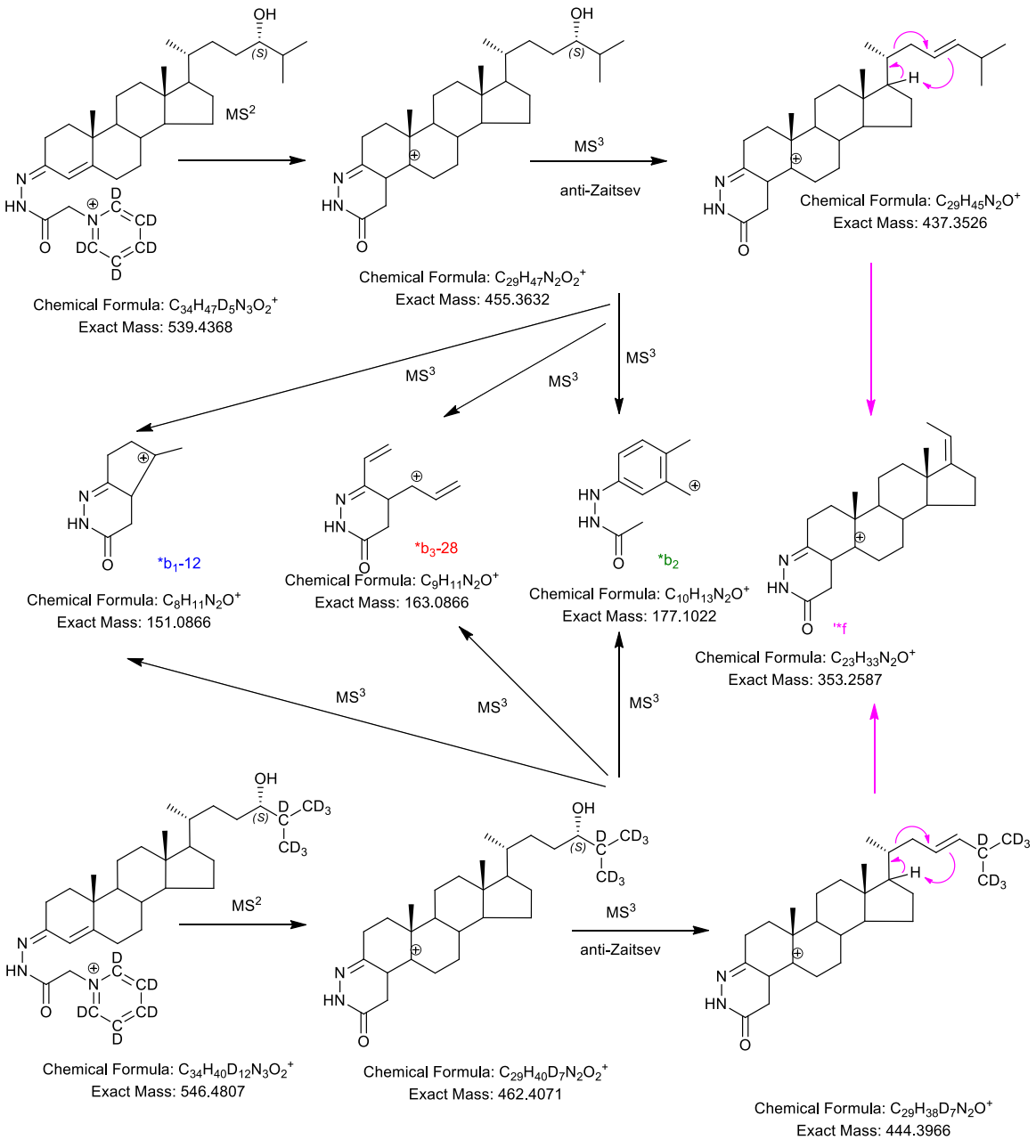
MS^3: 545→461→
18.35 min (37 min gradient)
$[^2H_6]$(25R)26-HC



Chemical Formula: $C_{34}H_{41}D_{11}N_3O_2^+$
Exact Mass: 545.4744

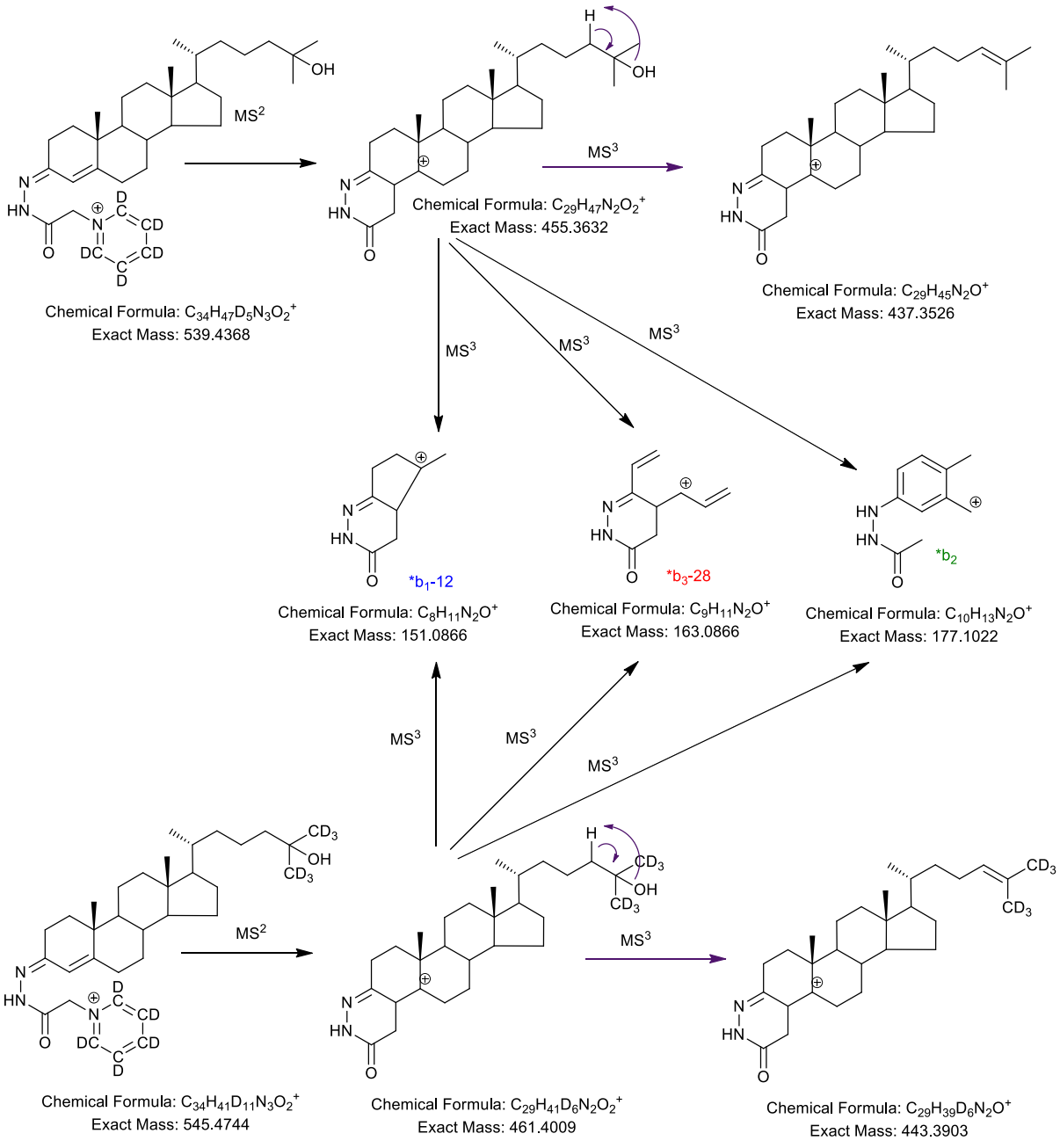
S4A

Figure S4A



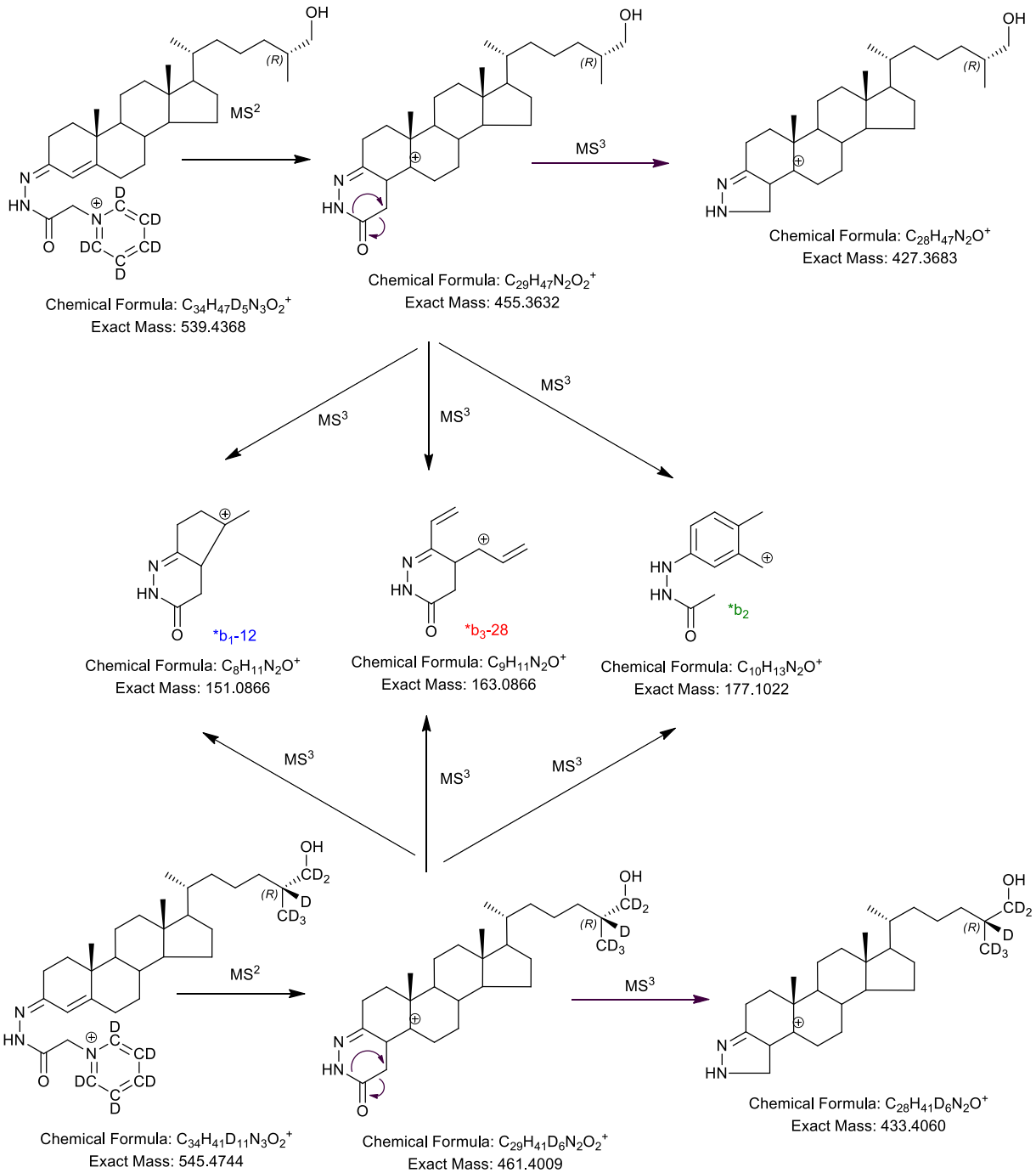
S4B

Figure S4B



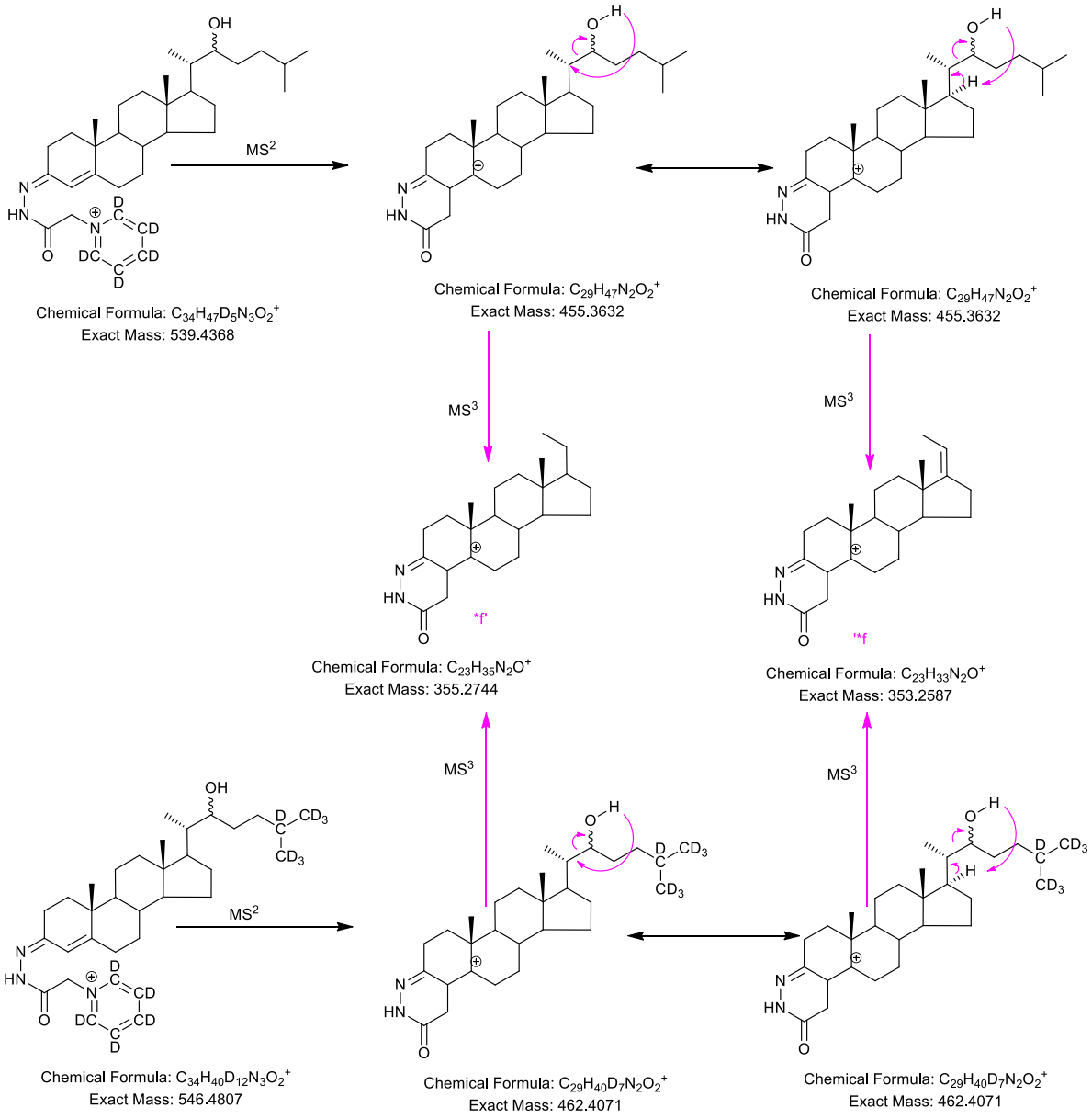
S4C

Figure S4C



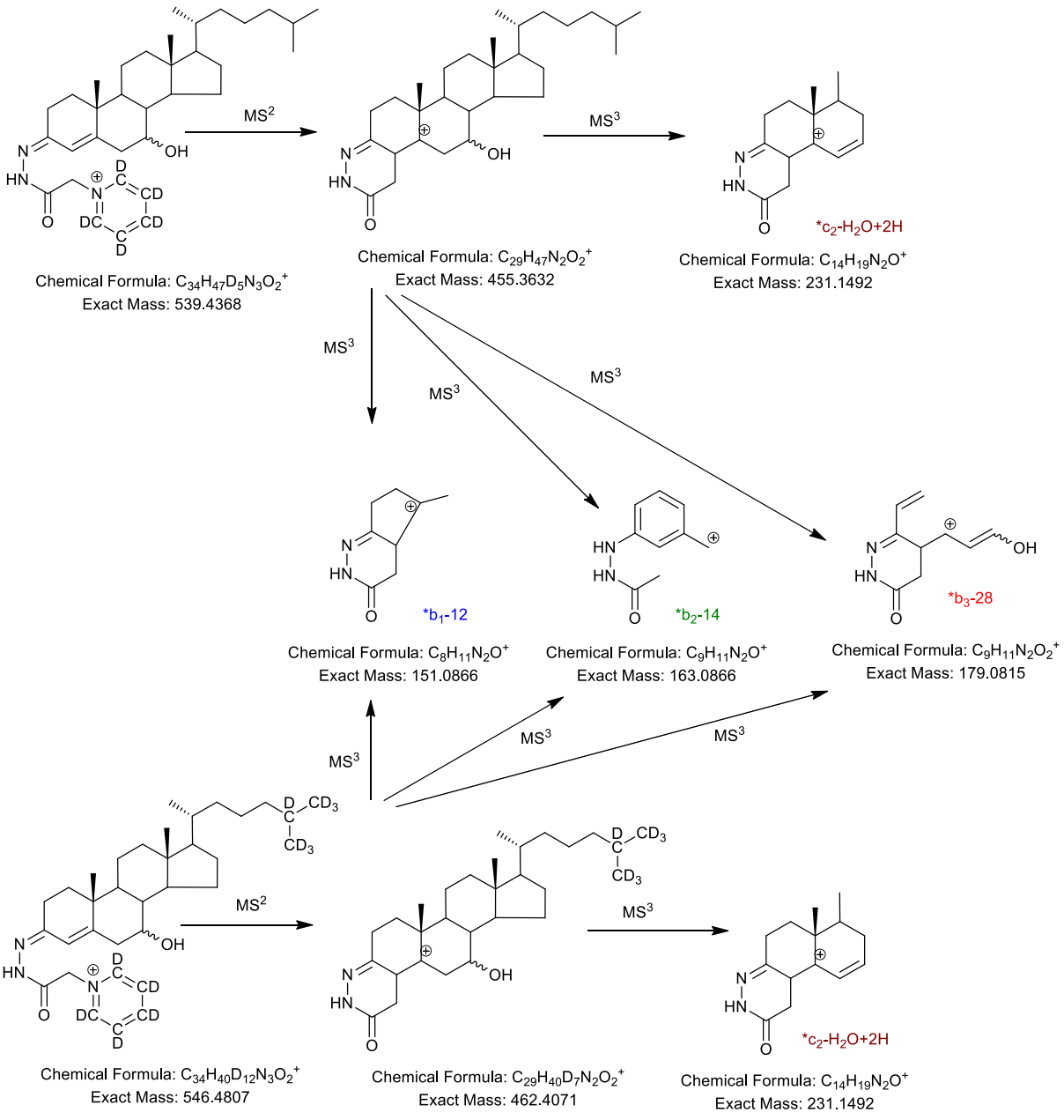
S4D

Figure 4D



S4E

Figure S4E



Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

Chemical Formula: $C_{29}H_{47}N_2O_2^+$
Exact Mass: 455.3632

Chemical Formula: $C_{14}H_{19}N_2O^+$
Exact Mass: 231.1492

Chemical Formula: $C_8H_{11}N_2O^+$
Exact Mass: 151.0866

Chemical Formula: $C_9H_{11}N_2O^+$
Exact Mass: 163.0866

Chemical Formula: $C_9H_{11}N_2O_2^+$
Exact Mass: 179.0815

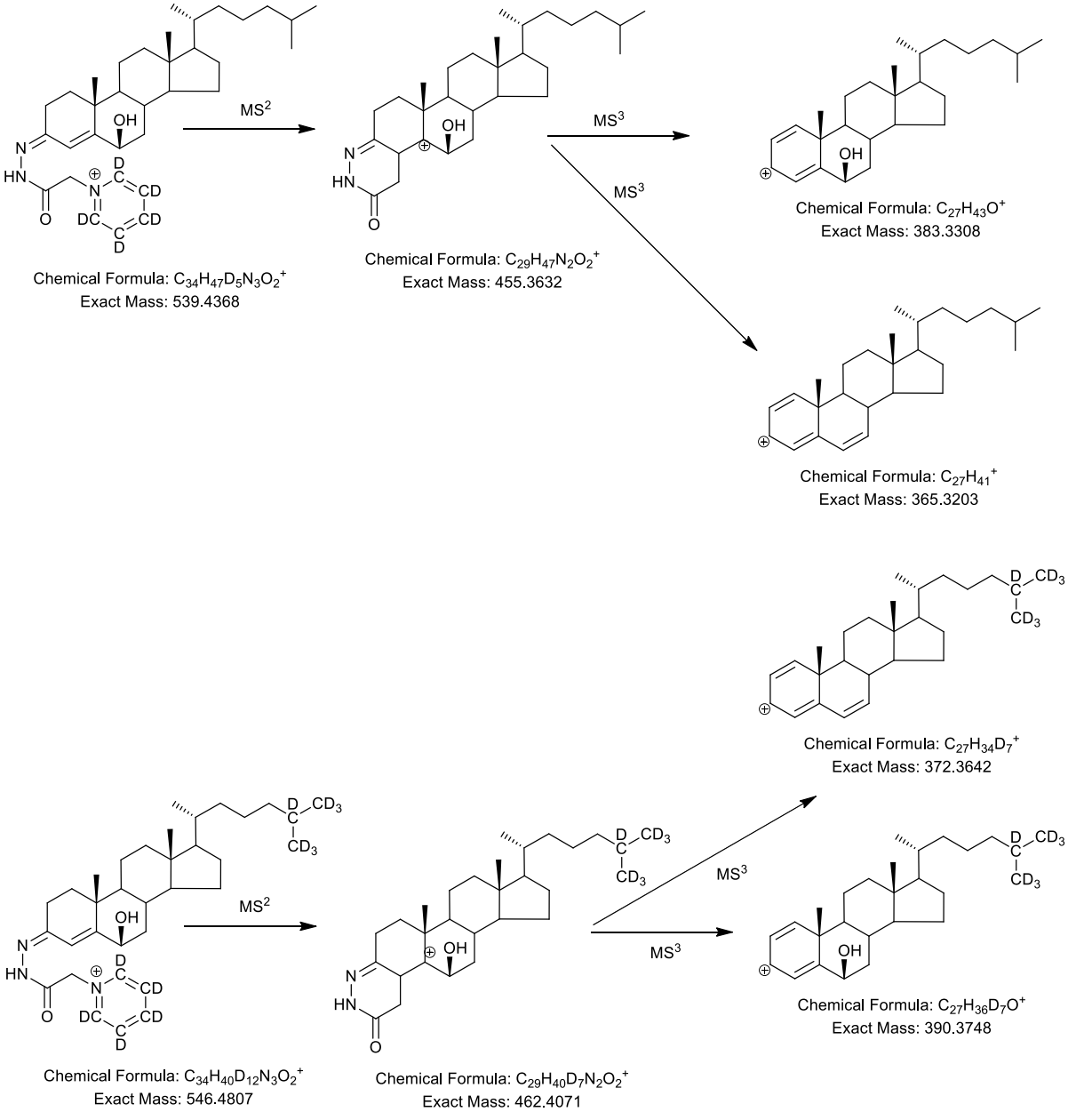
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

Chemical Formula: $C_{29}H_{40}D_7N_2O_2^+$
Exact Mass: 462.4071

Chemical Formula: $C_{14}H_{19}N_2O^+$
Exact Mass: 231.1492

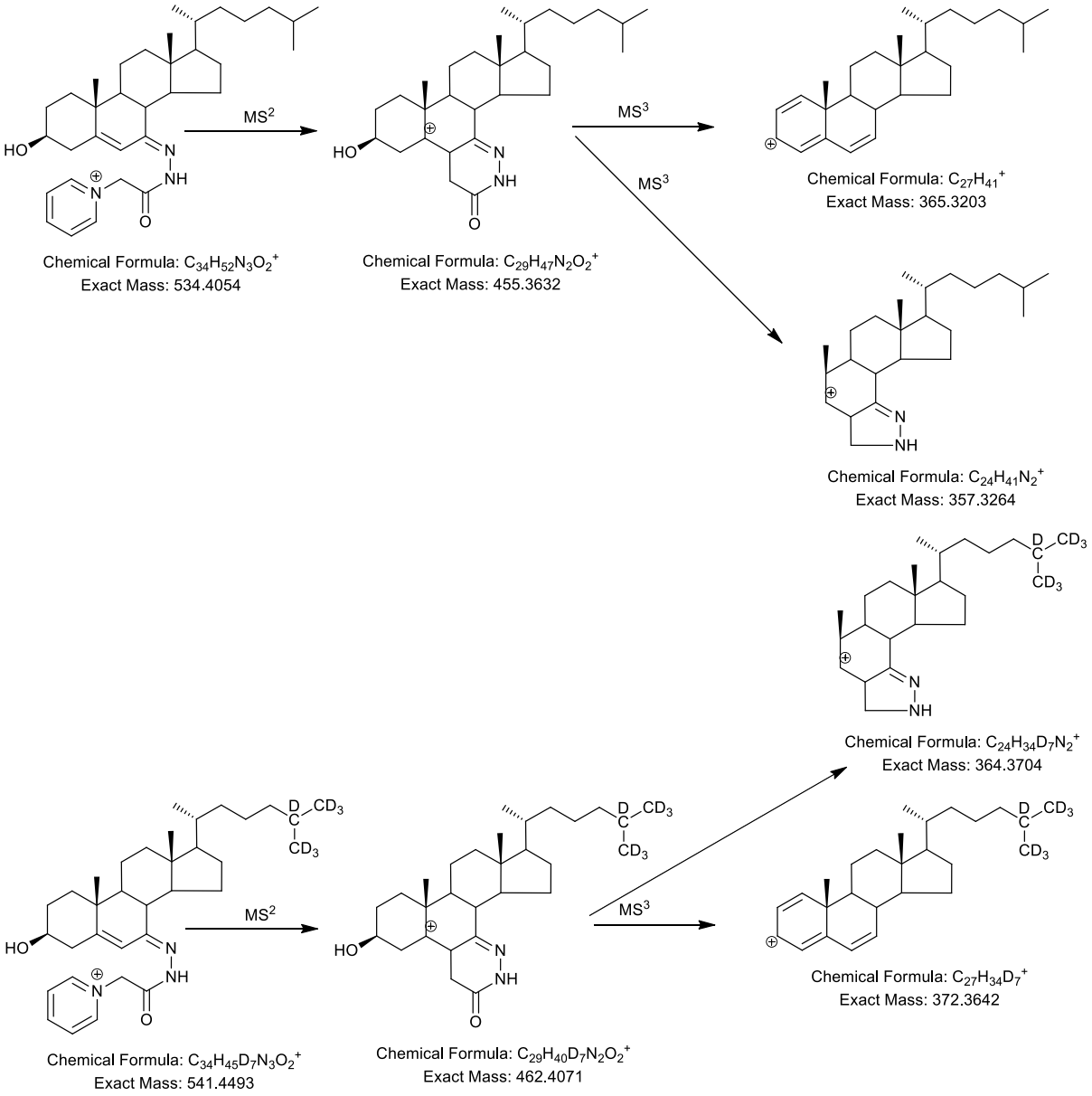
S4F

Figure S4F



S4G

Figure S4G



S4H

Figure S4H

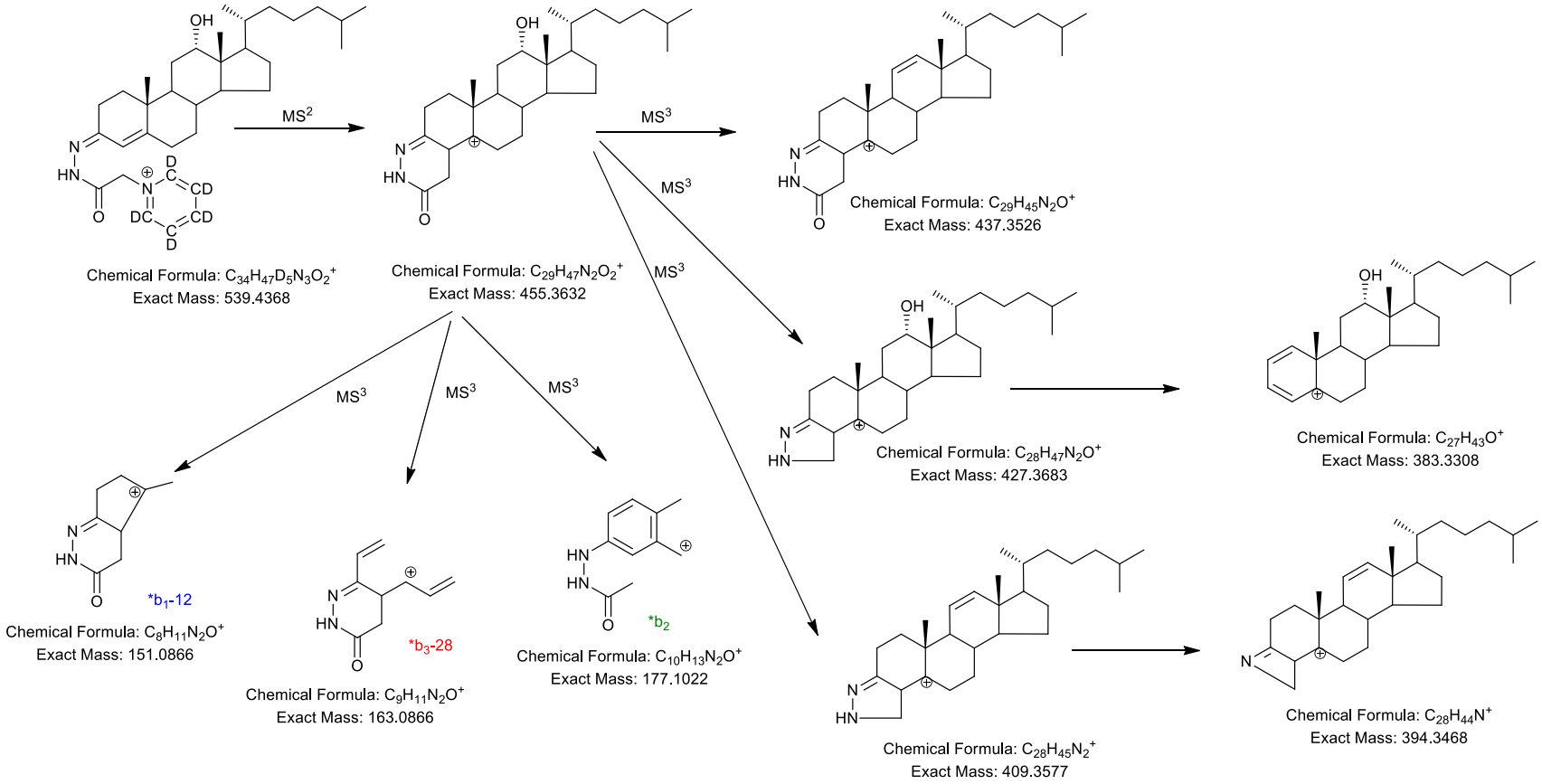
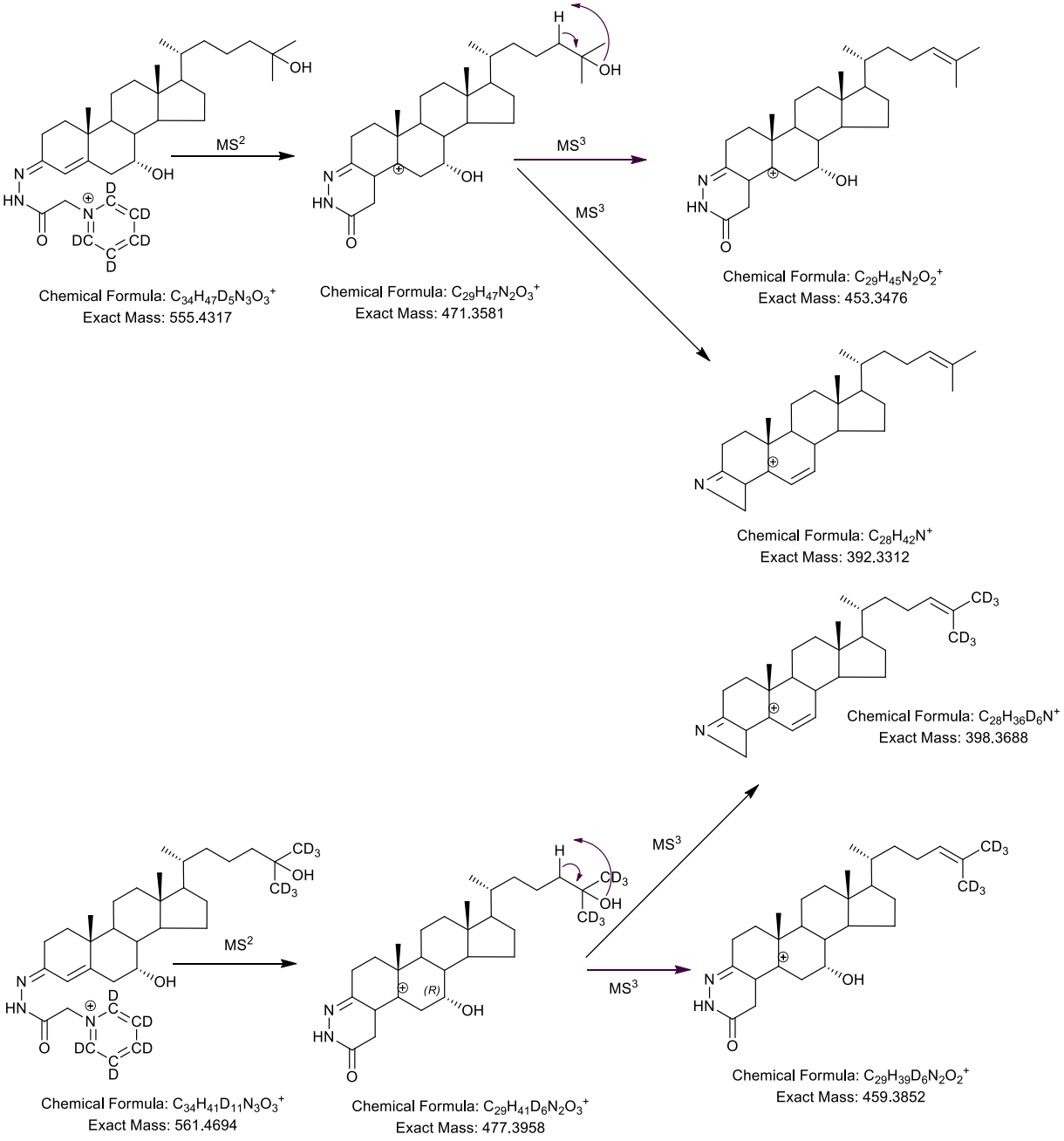
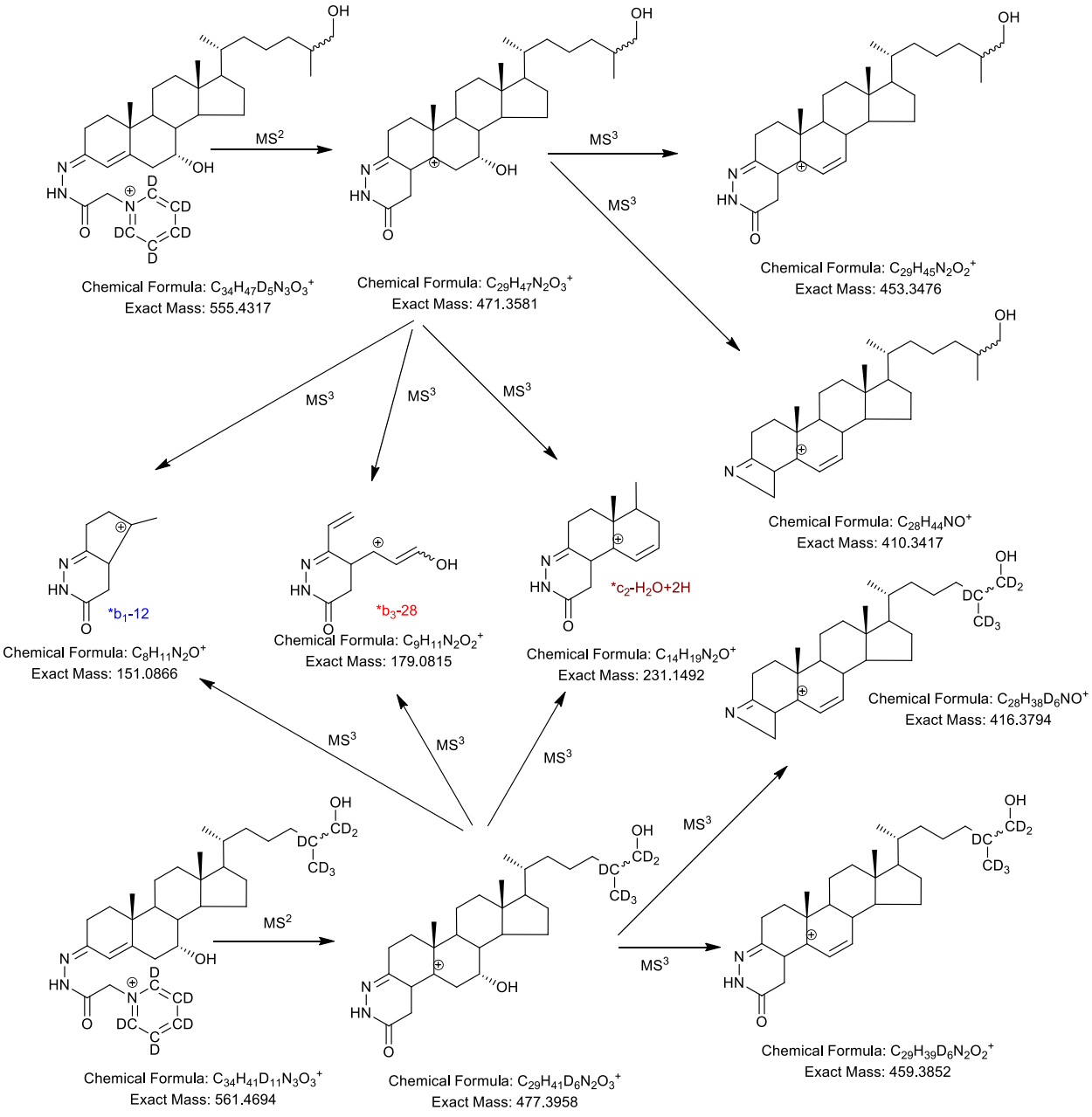


Figure S4I


MS2
MS3
MS3
MS3
MS2
MS3
(R)
Chemical Formula: C34H47D5N3O3+
Exact Mass: 555.4317
Chemical Formula: C29H47N2O3+
Exact Mass: 471.3581
Chemical Formula: C29H45N2O2+
Exact Mass: 453.3476
Chemical Formula: C28H42N+
Exact Mass: 392.3312
Chemical Formula: C28H36D6N+
Exact Mass: 398.3688
Chemical Formula: C34H41D11N3O3+
Exact Mass: 561.4694
Chemical Formula: C29H41D6N2O3+
Exact Mass: 477.3958
Chemical Formula: C29H39D6N2O2+
Exact Mass: 459.3852

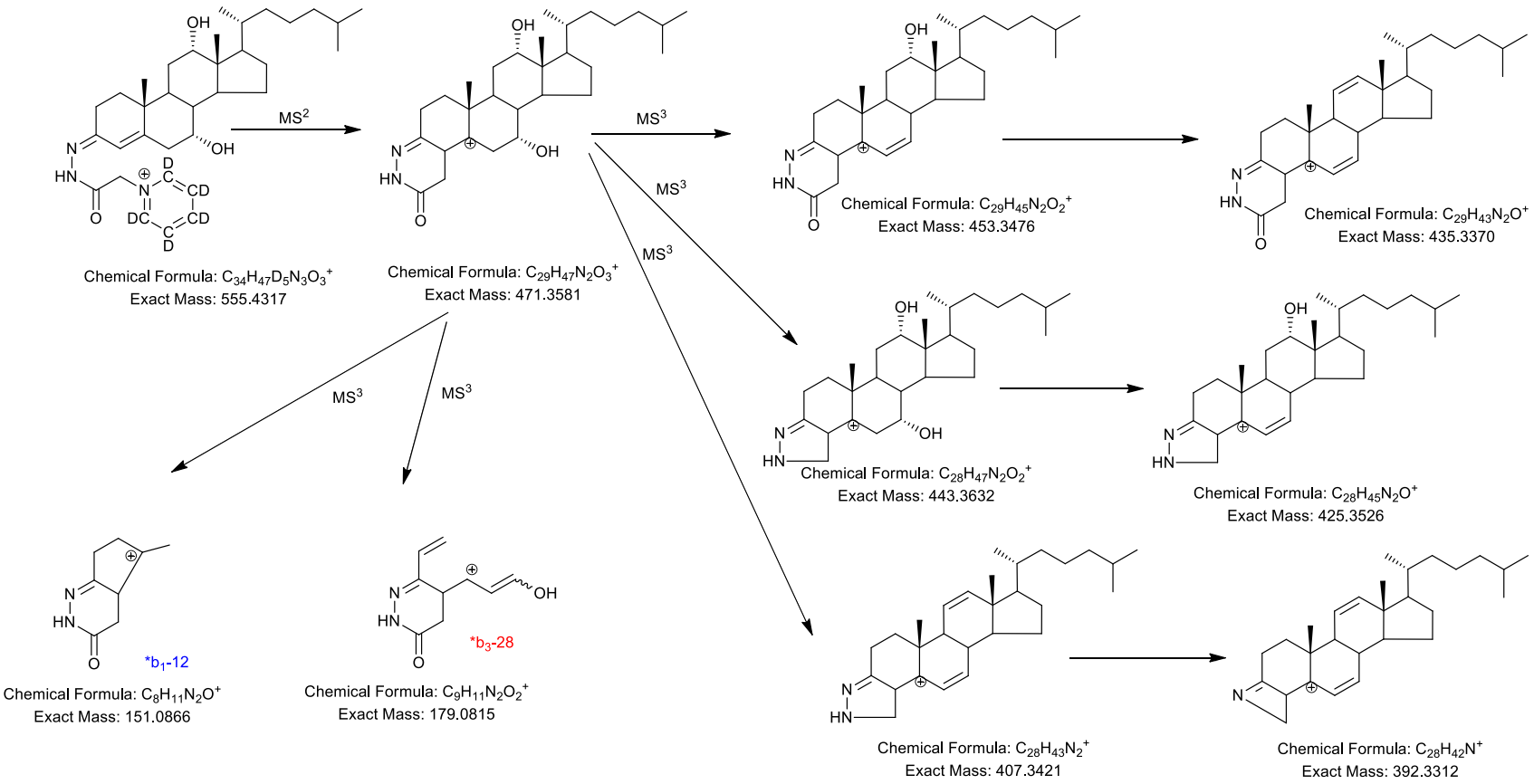
S4J

Figure S4J



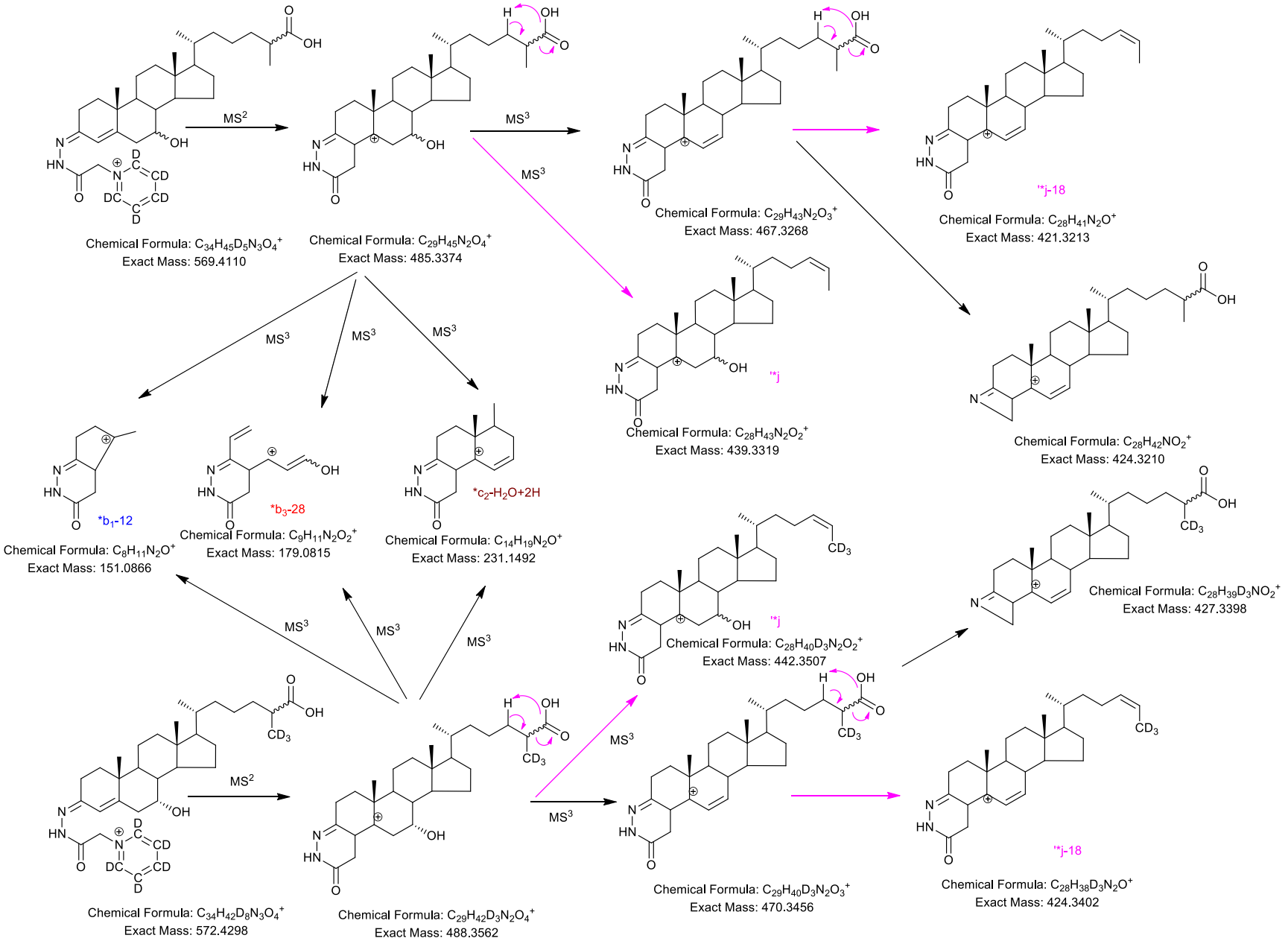
S4K

Figure S4K



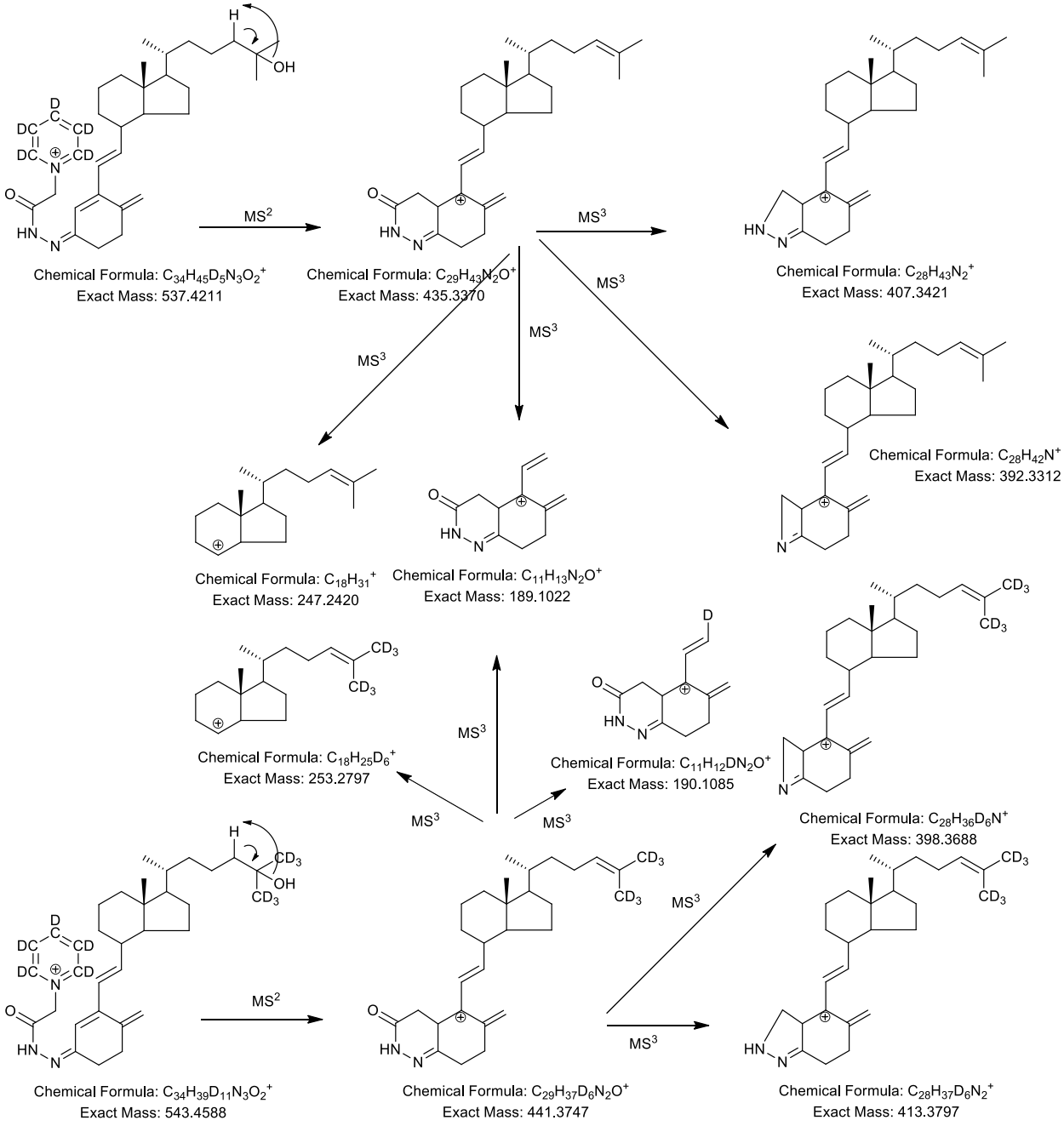
S4L

Figure S4L



S4M

Figure S4M



S4N

Figure S4N upper

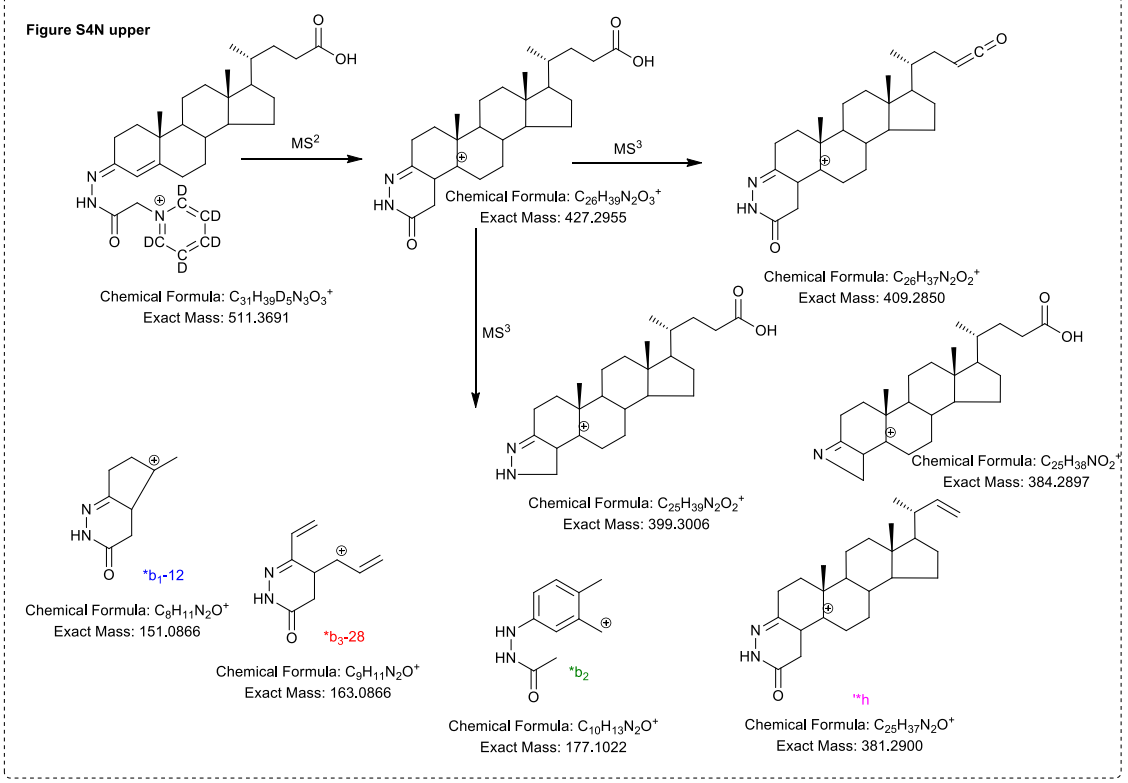
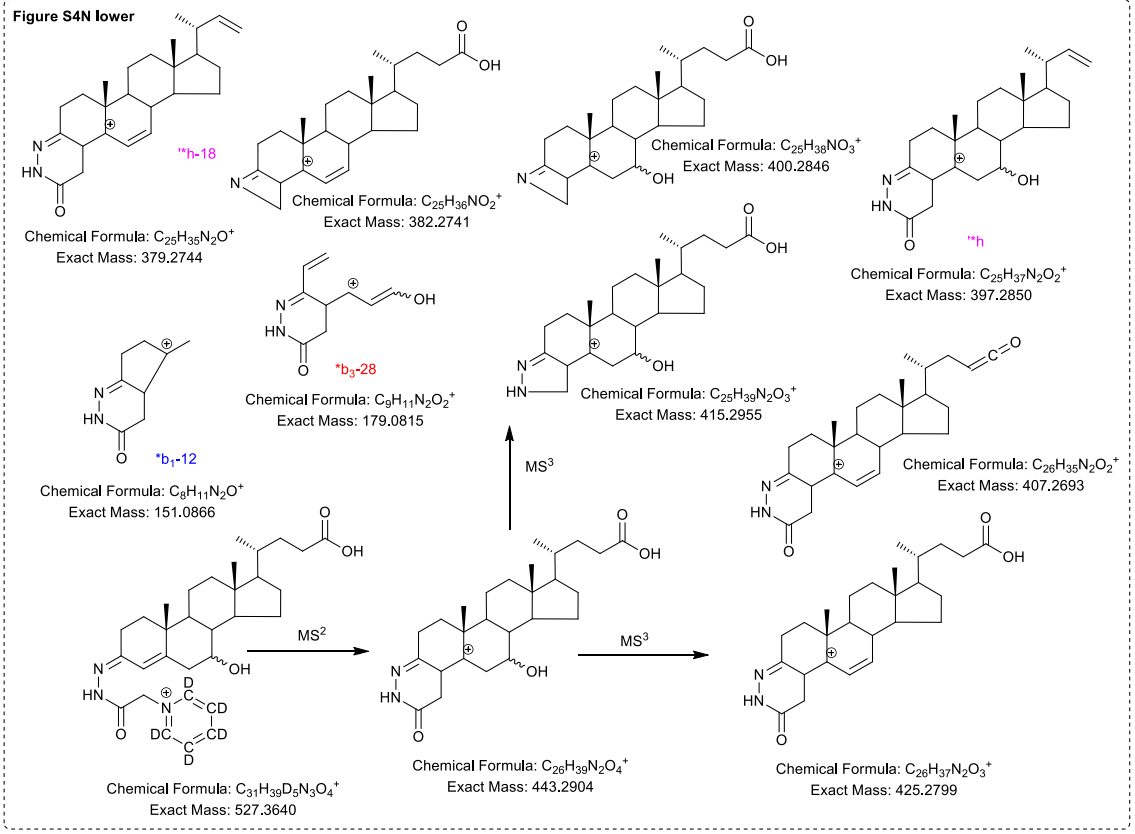
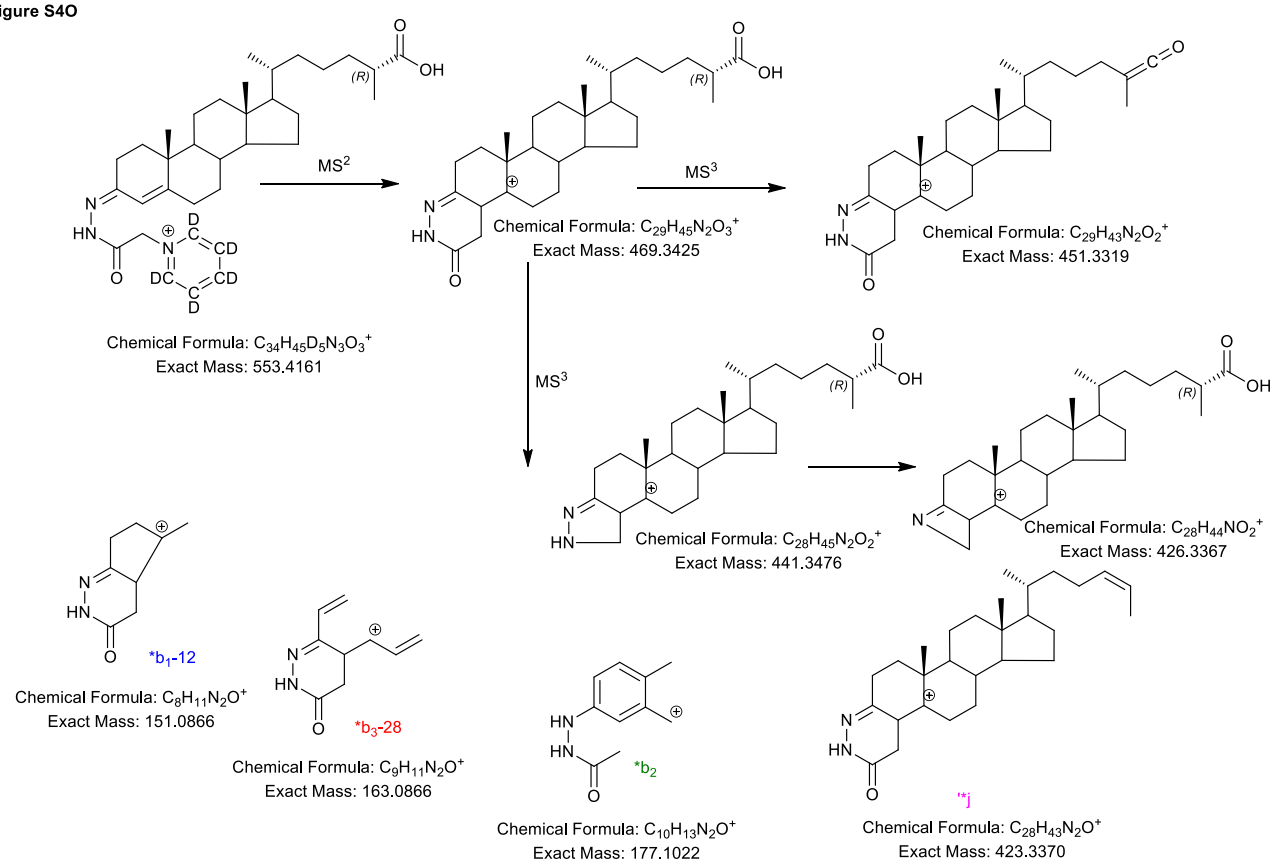


Figure S4N lower



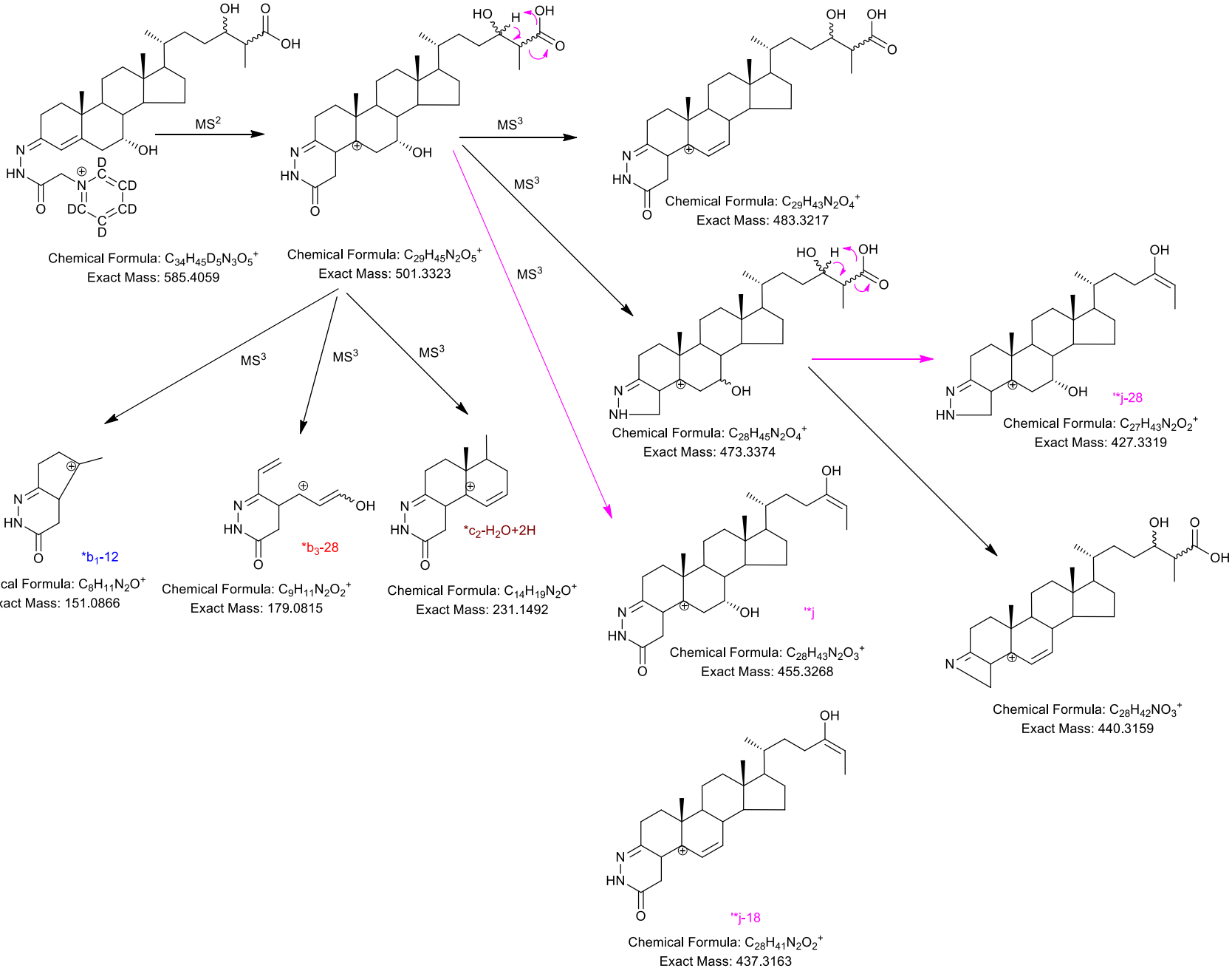
S4O

Figure S4O



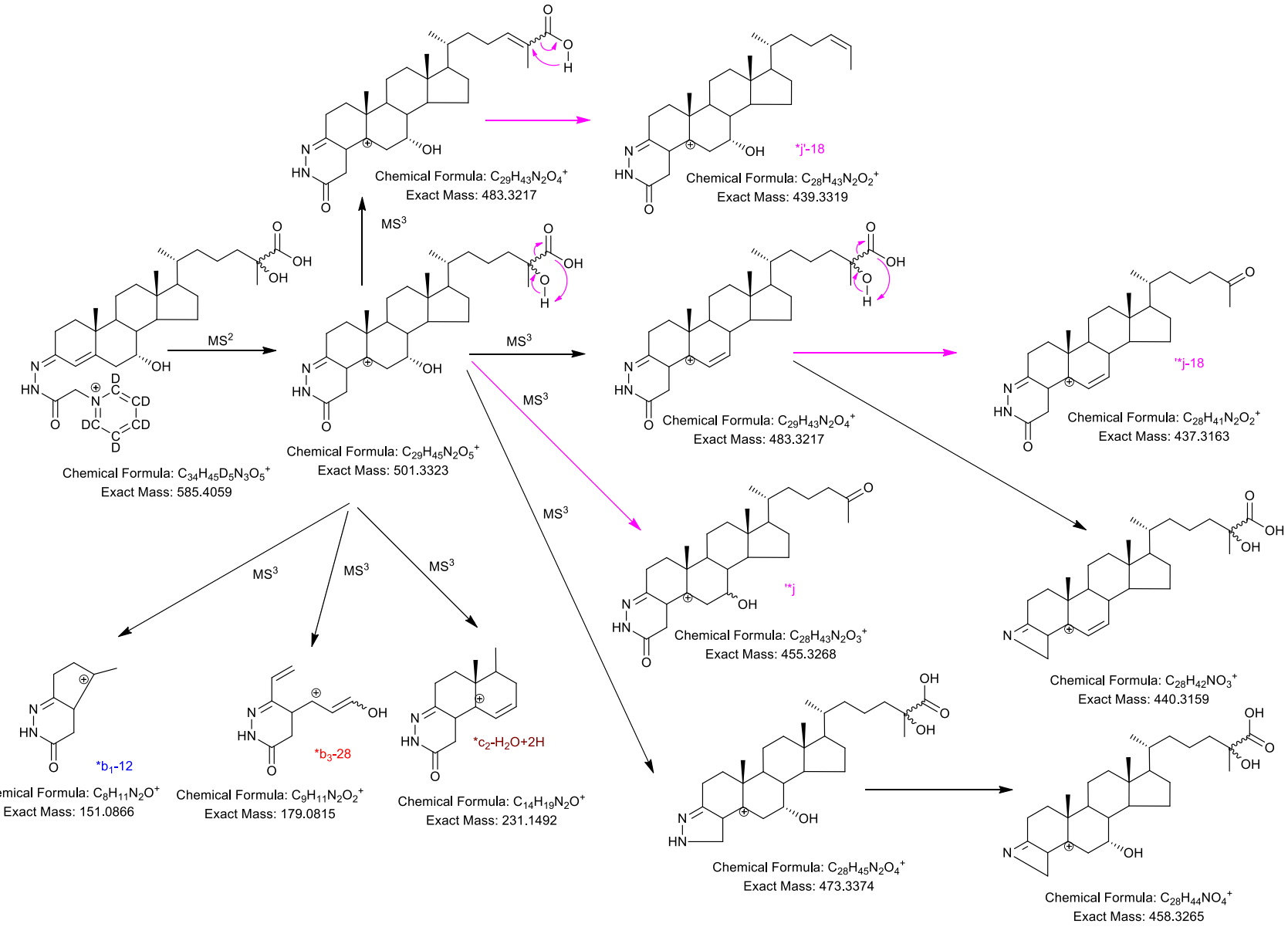
S4P

Figure 4P



S4Q

Figure 4Q



S4R

Figure S4R

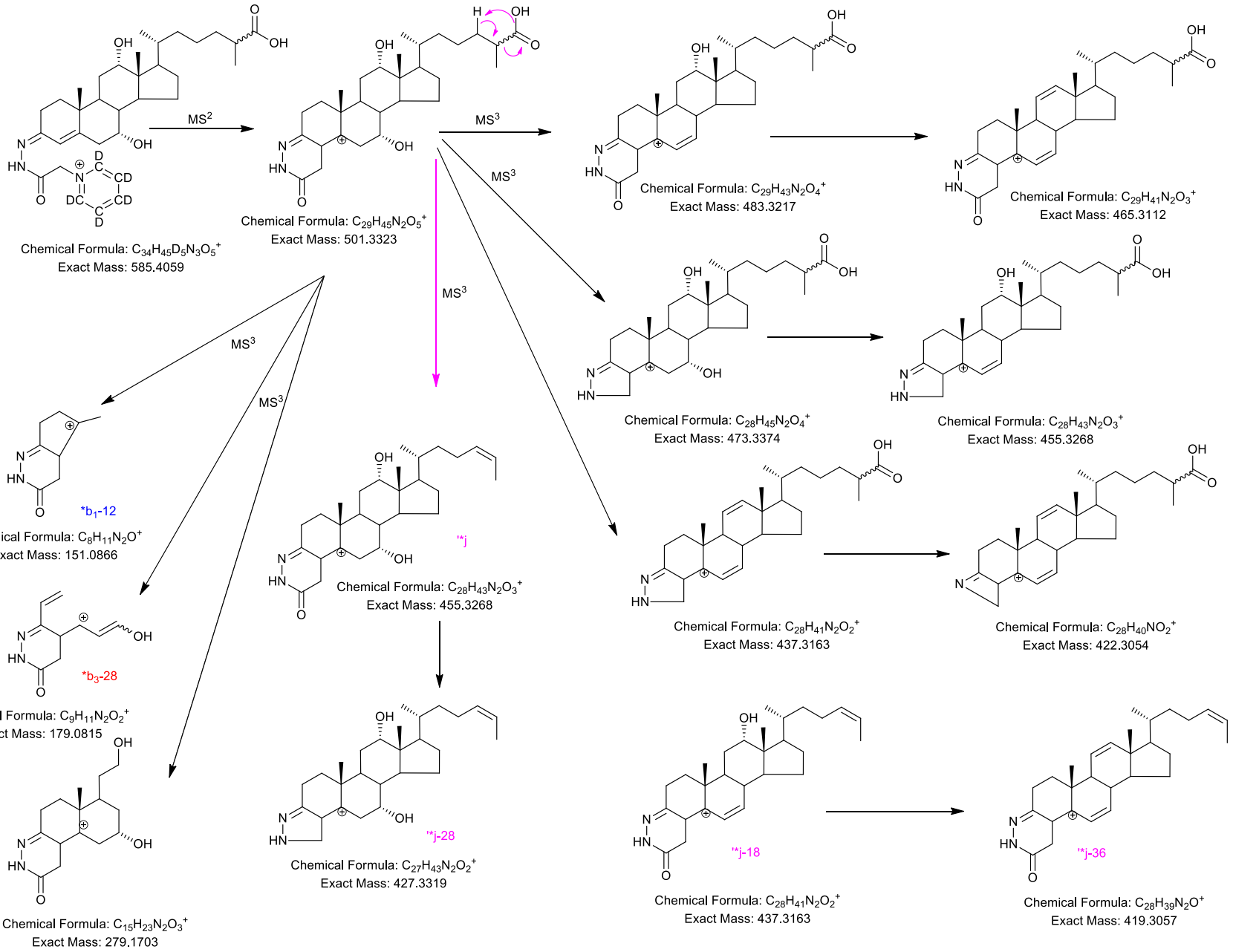
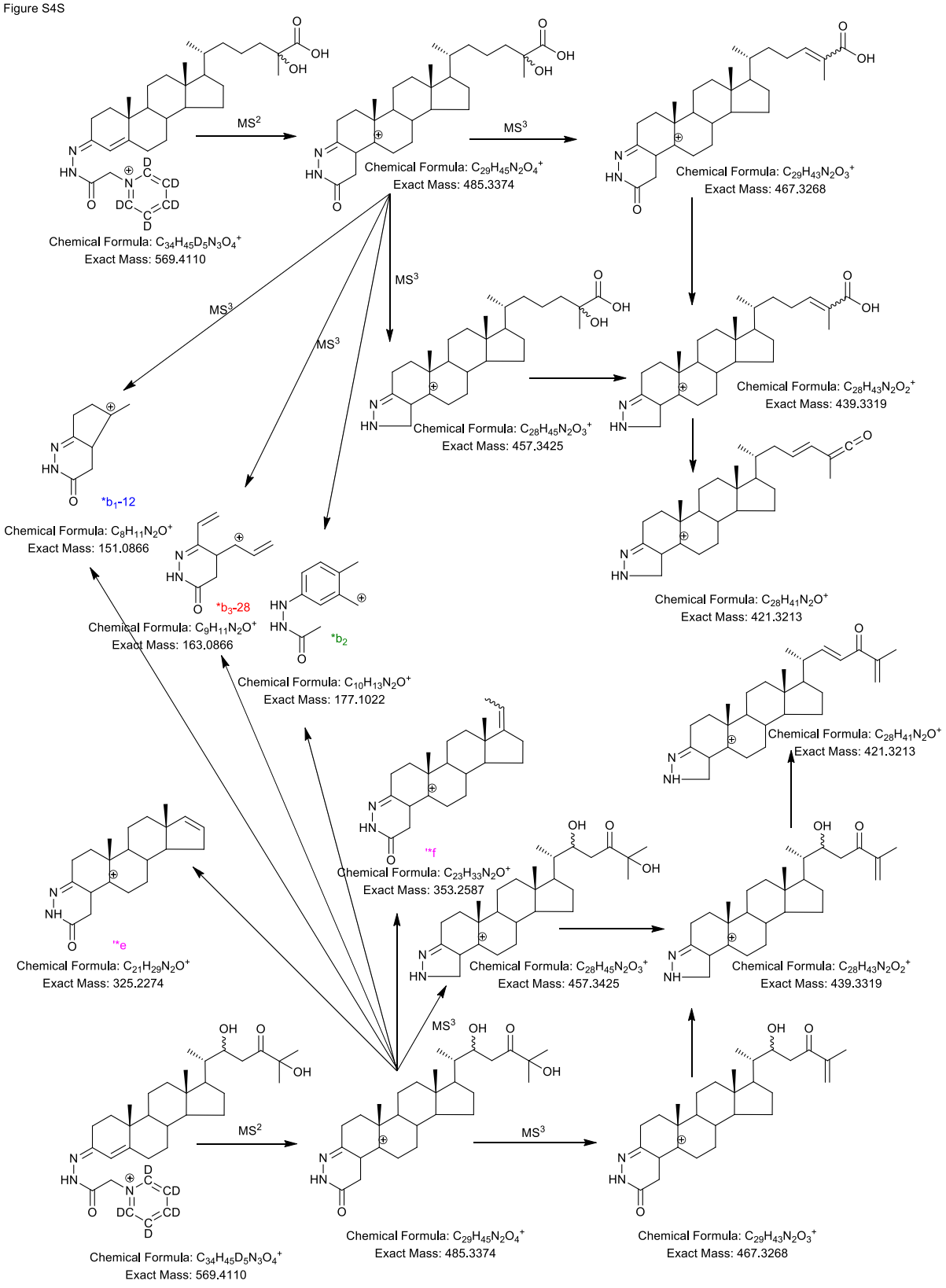
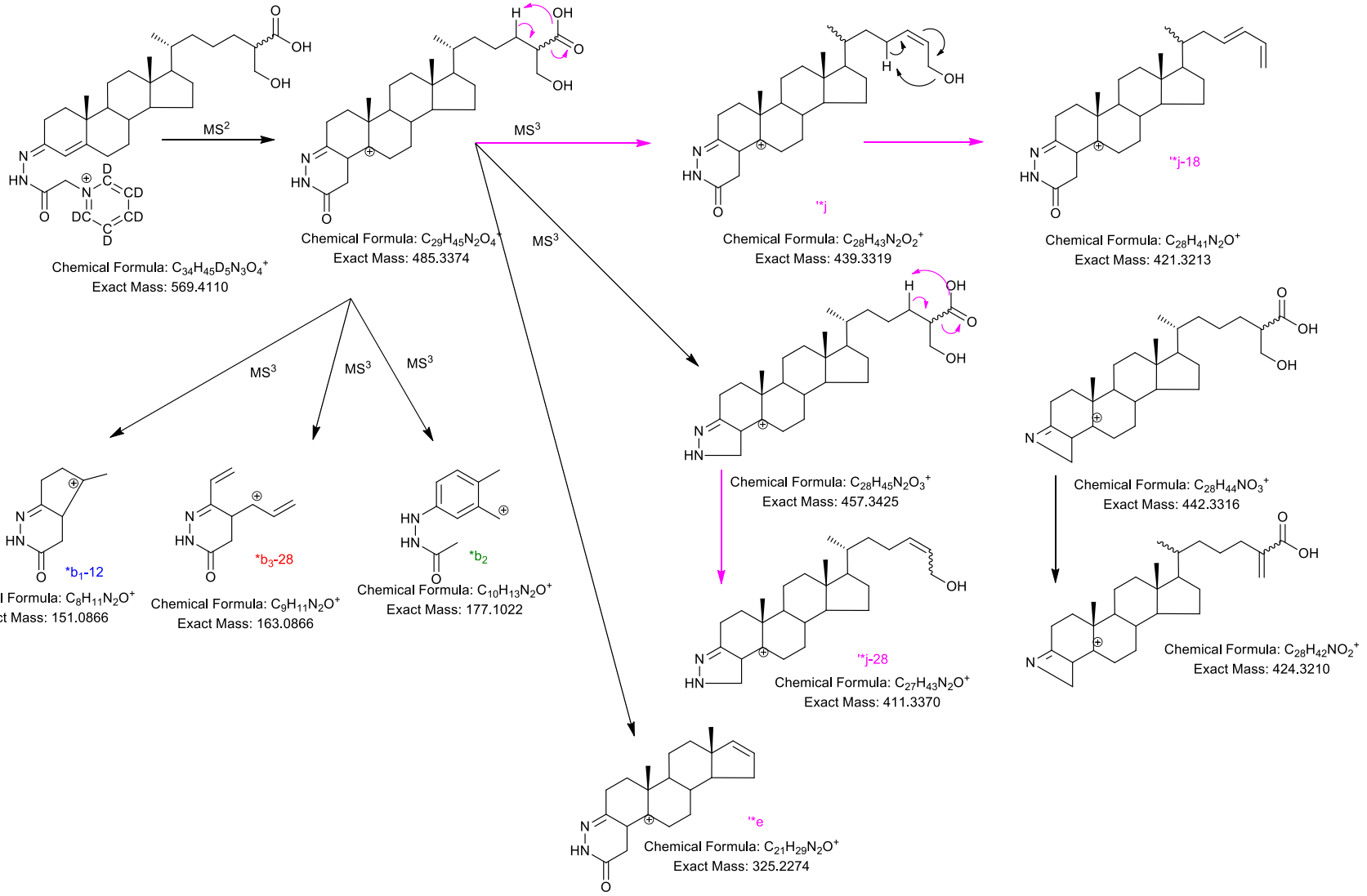


Figure S4S


MS2
MS3
MS3
MS3
MS3
MS3
MS2
MS3
Chemical Formula: C34H45D5N3O4+
Exact Mass: 569.4110
Chemical Formula: C29H45N2O4+
Exact Mass: 485.3374
Chemical Formula: C29H43N2O3+
Exact Mass: 467.3268
Chemical Formula: C28H45N2O3+
Exact Mass: 457.3425
Chemical Formula: C28H43N2O2+
Exact Mass: 439.3319
Chemical Formula: C28H41N2O+
Exact Mass: 421.3213
*b1-12
Chemical Formula: C8H11N2O+
Exact Mass: 151.0866
*b3-28
Chemical Formula: C9H11N2O+
Exact Mass: 163.0866
*b2
Chemical Formula: C10H13N2O+
Exact Mass: 177.1022
'*f
Chemical Formula: C23H33N2O+
Exact Mass: 353.2587
'*e
Chemical Formula: C21H29N2O+
Exact Mass: 325.2274
Chemical Formula: C28H41N2O+
Exact Mass: 421.3213
Chemical Formula: C28H45N2O3+
Exact Mass: 457.3425
Chemical Formula: C28H43N2O2+
Exact Mass: 439.3319
Chemical Formula: C34H45D5N3O4+
Exact Mass: 569.4110
Chemical Formula: C29H45N2O4+
Exact Mass: 485.3374
Chemical Formula: C29H43N2O3+
Exact Mass: 467.3268

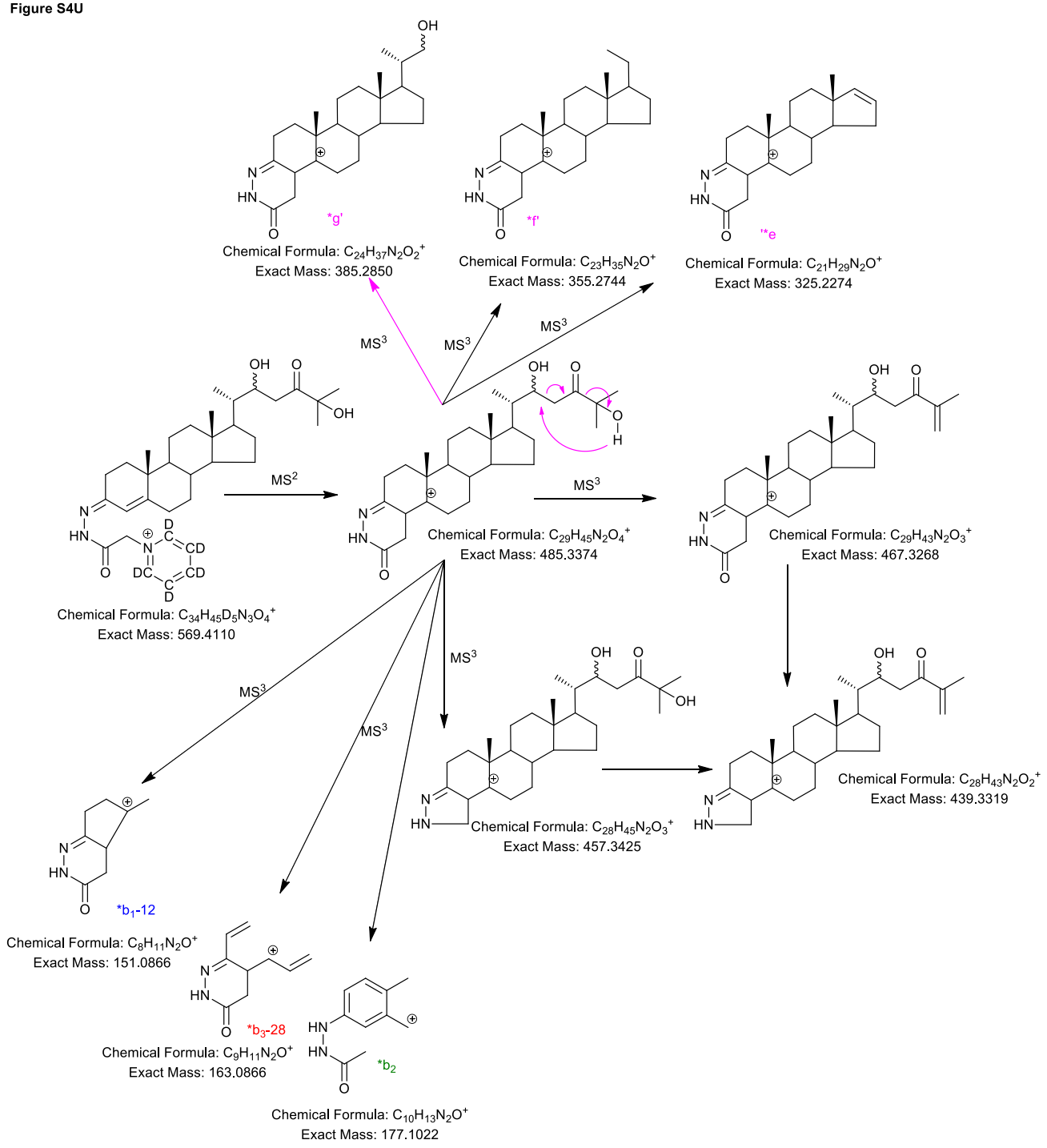
S4T

Figure S4T



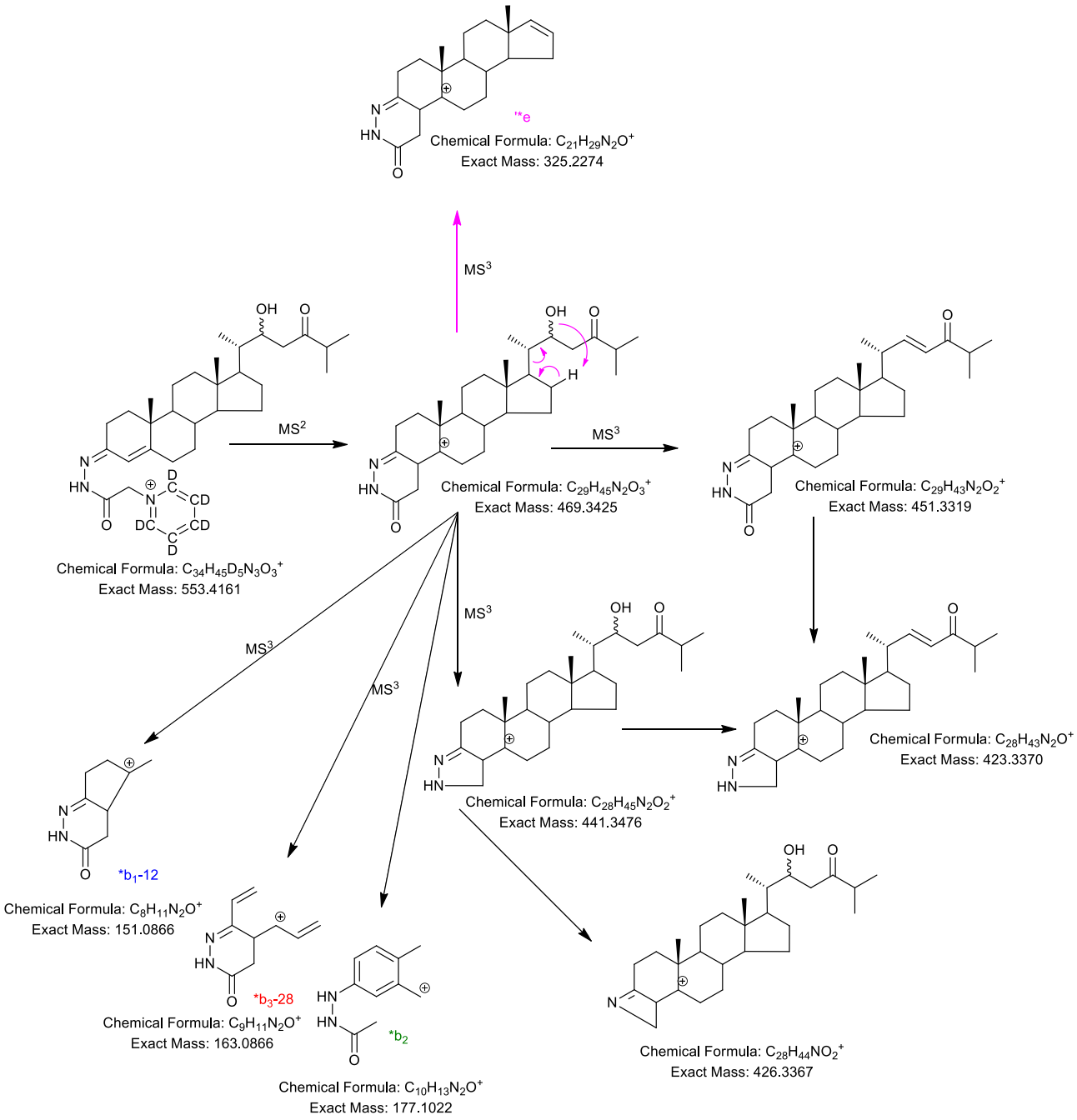
S4U

Figure S4U



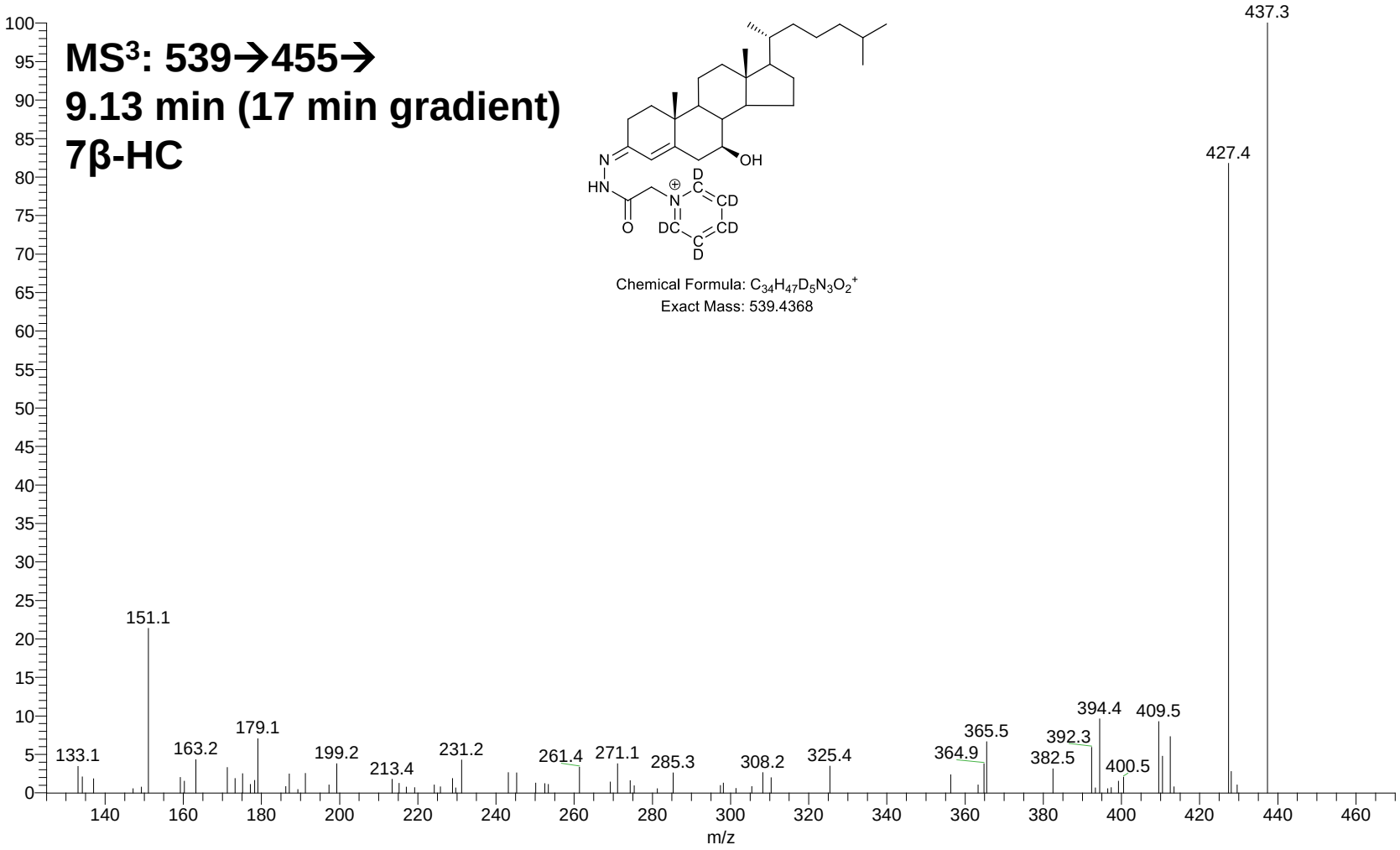
S4V

Figure S4V



S5A

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E 9.13 AV: 1 NL: 5.83E2
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

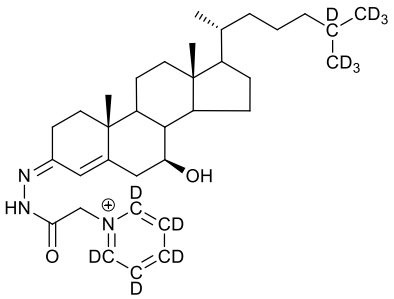


S5B

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

9.10 AV: 1 NL: 1.77E3

MS^3: 546→462→
9.13 min (17 min gradient)
$[^2H_7]$7β-HC



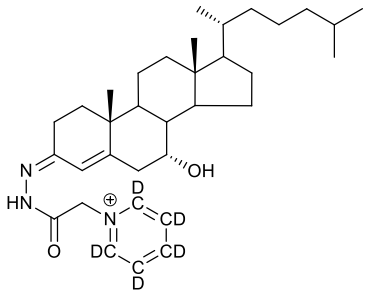
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

S5C

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

9.70 AV: 1 NL: 1.19E4

MS^3: 539→455→
9.70 min (17 min gradient)
7α-HC

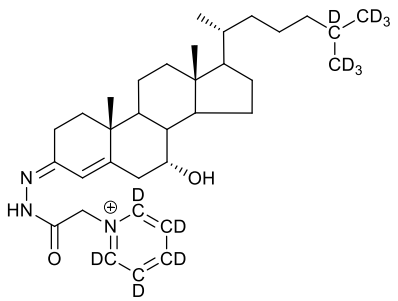


Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

S5D

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E 9.66 AV: 1 NL: 2.06E4
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

MS^3: 546→462→
9.66 min (17 min gradient)
$[^2H_7]$7α-HC



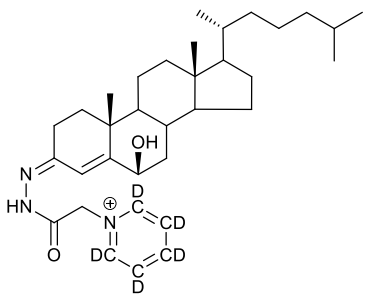
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

S5E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

9.97 AV: 1 NL: 1.11E3

MS³: 539→455→
9.97 min (17 min gradient)
6β-HC



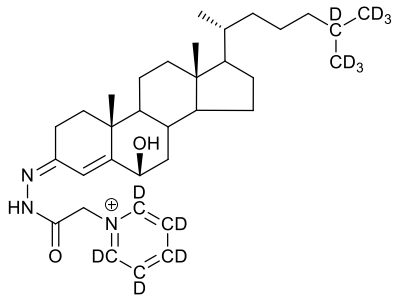
Chemical Formula: $C_{34}H_{47}D_5N_3O_2^+$
Exact Mass: 539.4368

S5F

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 546.48@cid30.00 462.41@cid35.00 [125.00-555.0

9.93 AV: 1 NL: 2.25E3

MS³: 546→462→
9.93 min (17 min gradient)
[²H₇]6β-HC



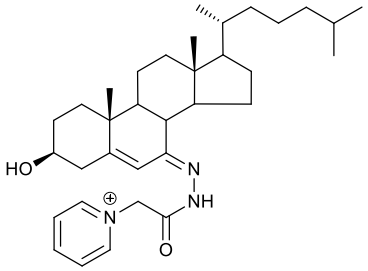
Chemical Formula: $C_{34}H_{40}D_{12}N_3O_2^+$
Exact Mass: 546.4807

S5G

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_F
F: ITMS + c ESI Full ms3 534.41@cid30.00 455.36@cid35.00 [125.00-55

T: 9.37 AV: 1 NL: 1.07E3

MS^3: 534→455→
9.37 min (17 min gradient)
7-OC



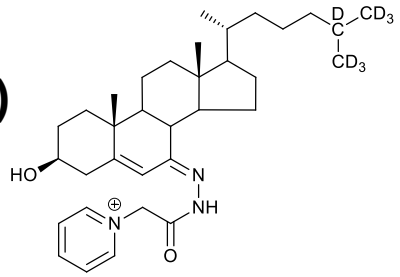
Chemical Formula: $C_{34}H_{52}N_3O_2^+$
Exact Mass: 534.4054

S5H

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_F
F: ITMS + c ESI Full ms3 541.45@cid30.00 462.41@cid35.00 [125.00-55

T: 9.33 AV: 1 NL: 2.41E3

MS^3: 541→462→
9.33 min (17 min gradient)
[$^2H_7$]7-OC



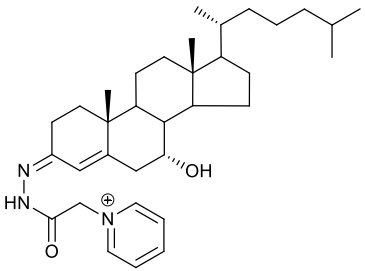
Chemical Formula: $C_{34}H_{45}D_7N_3O_2^+$
Exact Mass: 541.4493

S5I

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_F
F: ITMS + c ESI Full ms3 534.41@cid30.00 455.36@cid35.00 [125.00-55

T: 9.69 AV: 1 NL: 9.01E3

MS^3: 534→455→
9.69 min (17 min gradient)
7α-HCO



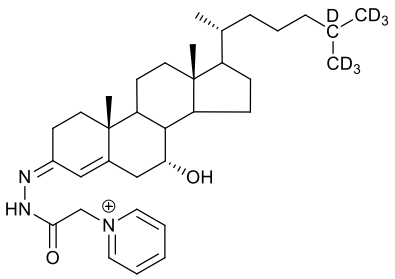
Chemical Formula: $C_{34}H_{52}N_3O_2^+$
Exact Mass: 534.4054

S5J

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_F
F: ITMS + c ESI Full ms3 541.45@cid30.00 462.41@cid35.00 [125.00-55

T: 9.65 AV: 1 NL: 6.05E3

MS³: 541→462→
9.65 min (17 min gradient)
[$^2H_7$]7α-HCO

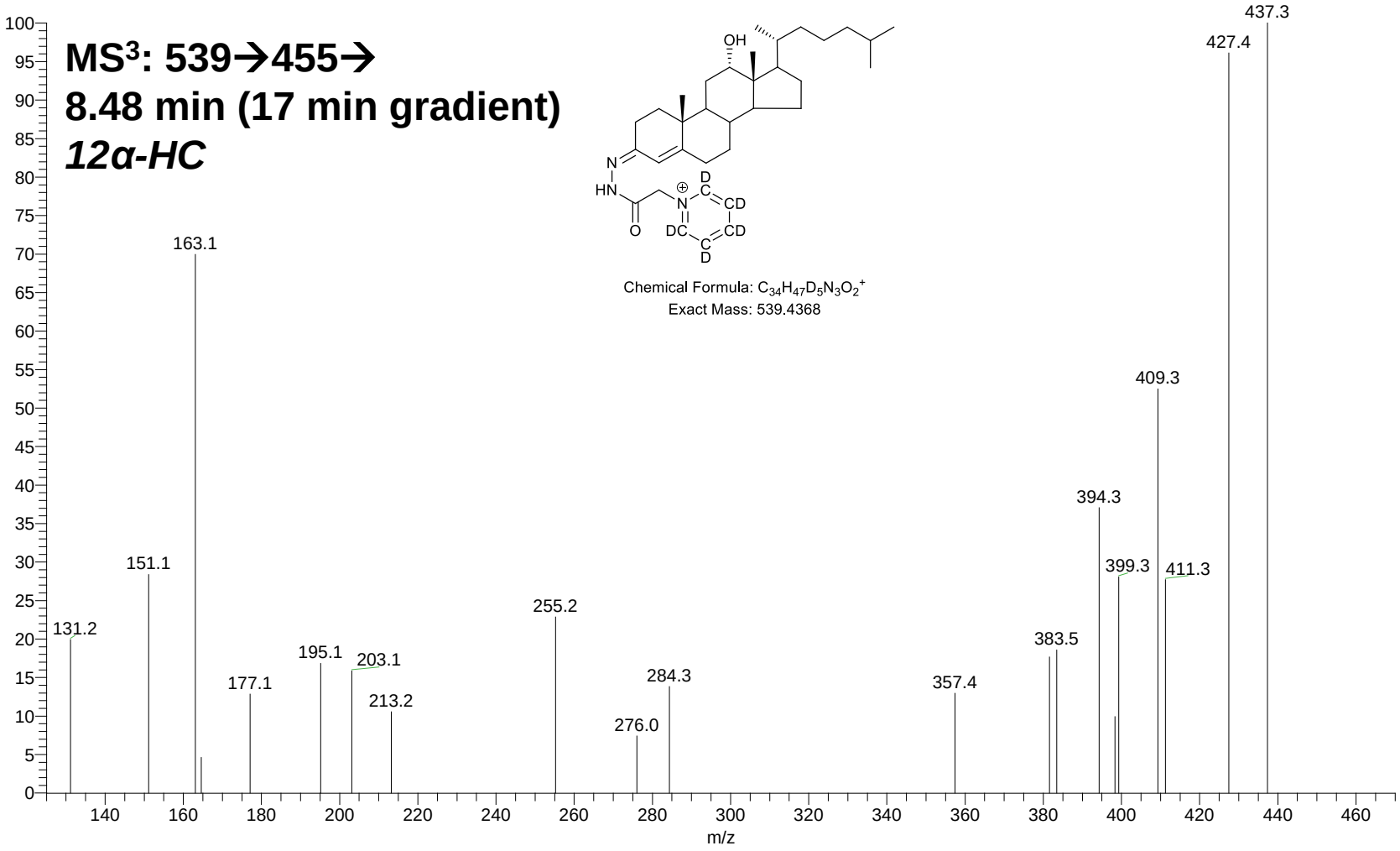


Chemical Formula: $C_{34}H_{45}D_7N_3O_2^+$
Exact Mass: 541.4493

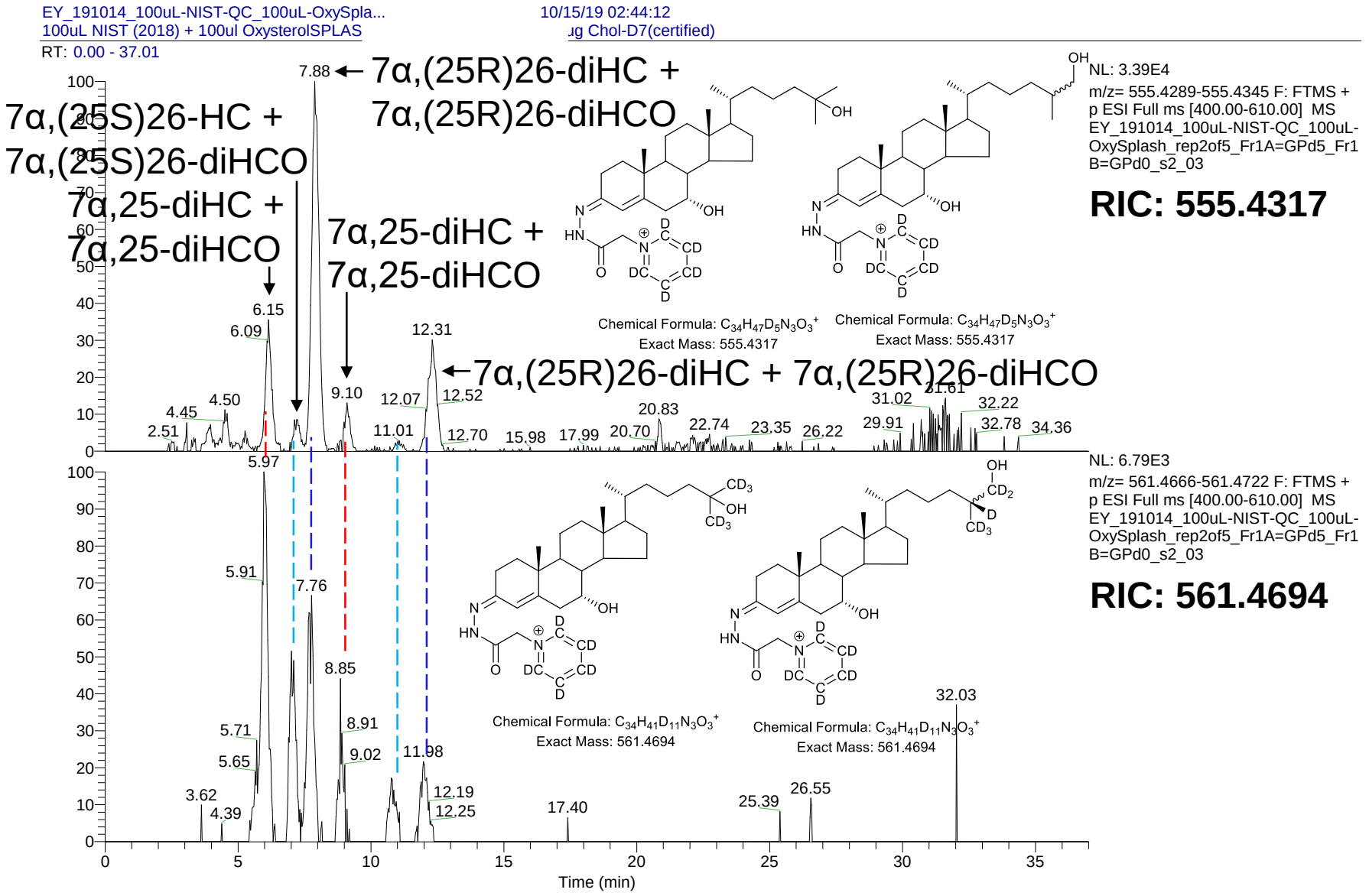
S5K

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 02:23:26
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 539.44@cid30.00 455.36@cid35.00 [125.00-550.0

8.48 AV: 1 NL: 5.96E1



S6A

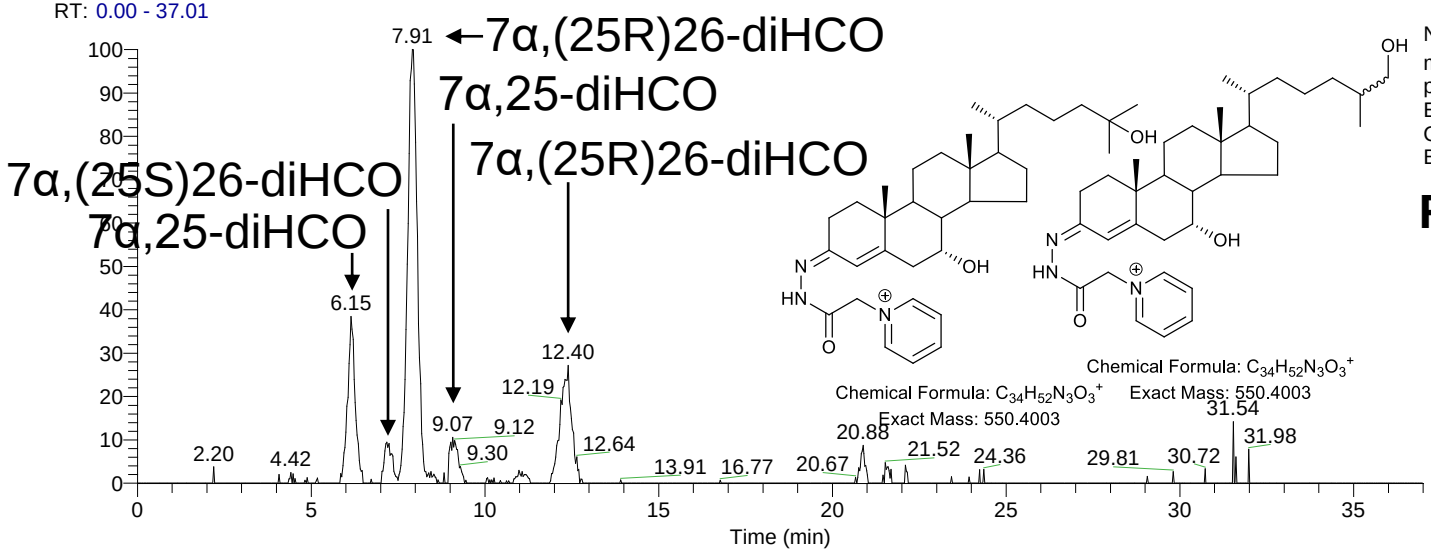

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS
10/15/19 02:44:12
ıg Chol-D7(certified)
RT: 0.00 - 37.01
7α,(25S)26-HC +
7α,(25S)26-diHCO
7α,25-diHC +
7α,25-diHCO
7.88 ← 7α,(25R)26-diHC +
7α,(25R)26-diHCO
7α,25-diHC +
7α,25-diHCO
←7α,(25R)26-diHC + 7α,(25R)26-diHCO
Chemical Formula: C34H47D5N3O3+
Exact Mass: 555.4317
Chemical Formula: C34H47D5N3O3+
Exact Mass: 555.4317
NL: 3.39E4
m/z= 555.4289-555.4345 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03
RIC: 555.4317
Chemical Formula: C34H41D11N3O3+
Exact Mass: 561.4694
Chemical Formula: C34H41D11N3O3+
Exact Mass: 561.4694
NL: 6.79E3
m/z= 561.4666-561.4722 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03
RIC: 561.4694
Time (min)

S6B

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPL

10/15/19 02:44:12
20ug Chol-D7(certified)

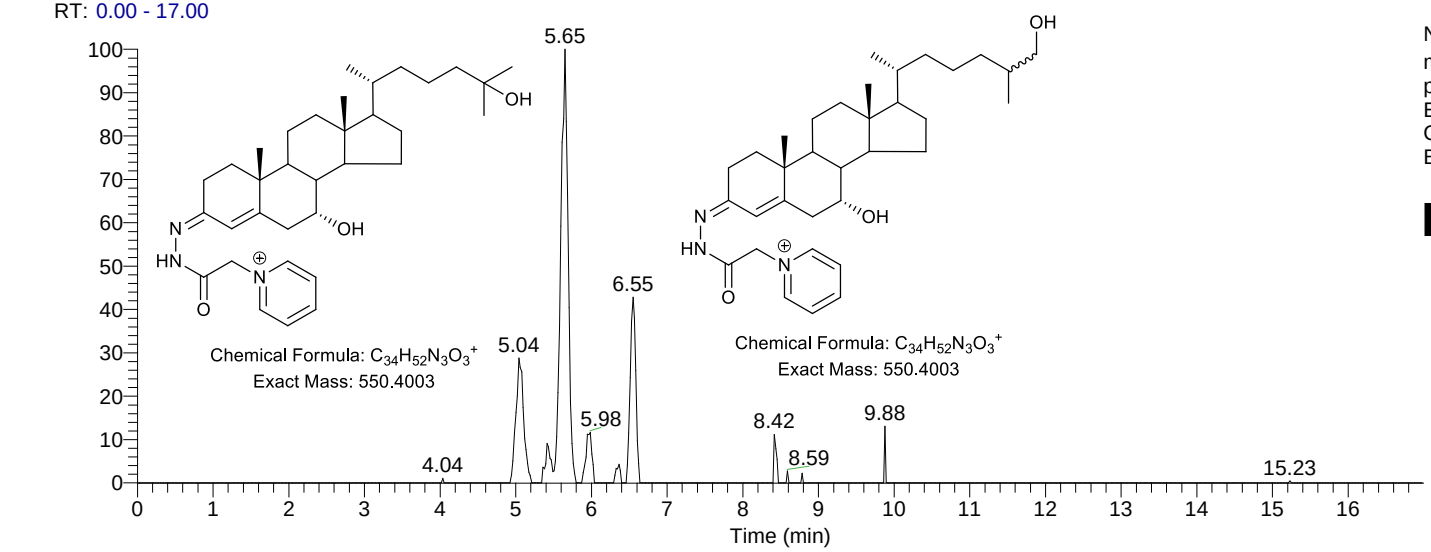
RT: 0.00 - 37.01



NL: 2.77E4
m/z= 550.3975-550.4031 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03

RIC: 550.4003

RT: 0.00 - 17.00



NL: 3.82E4
m/z= 550.3975-550.4031 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_04

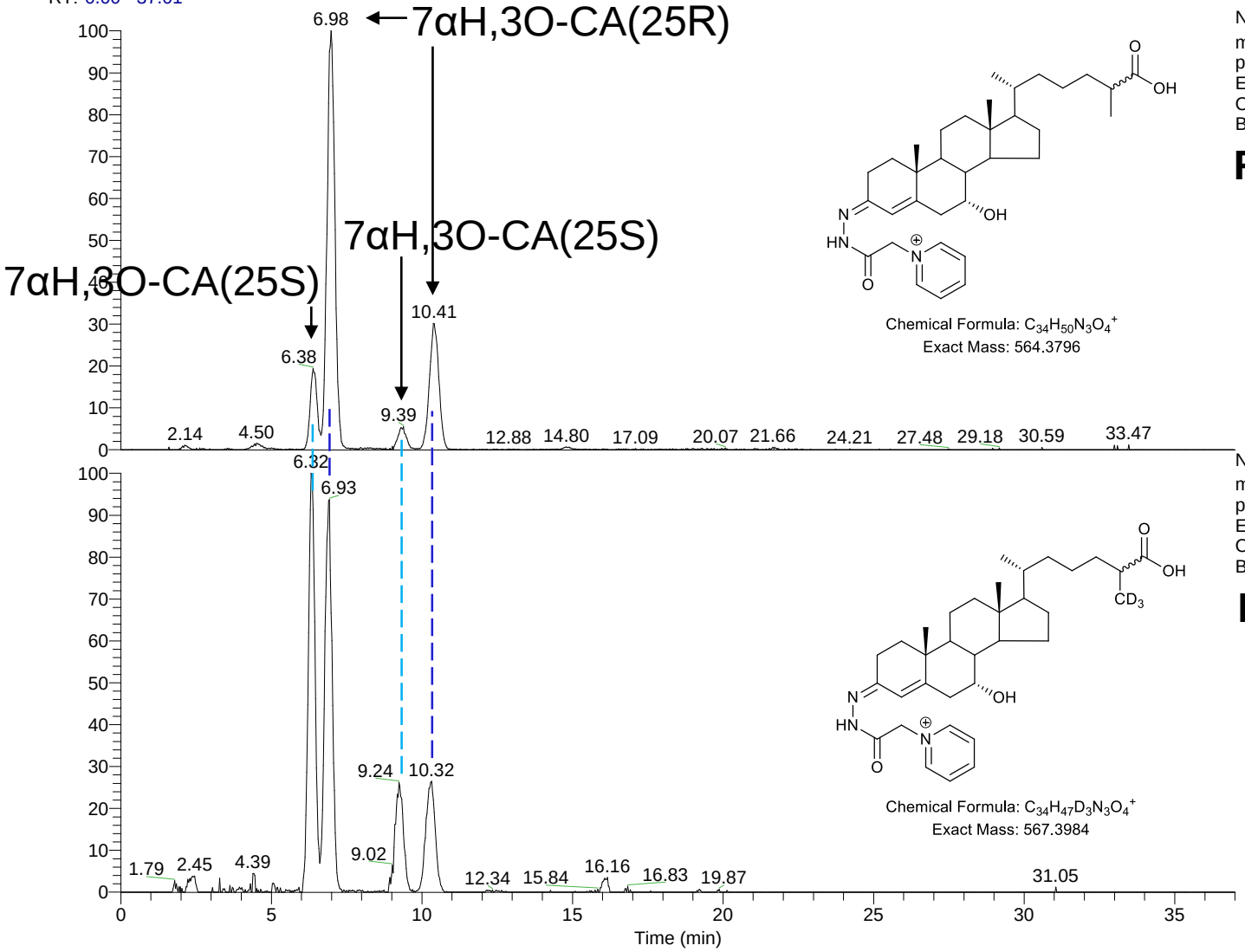
RIC: 550.4003

S6C

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS

10/15/19 02:44:12
ɹg Chol-D7(certified)

RT: 0.00 - 37.01



NL: 2.51E5
m/z= 564.3768-564.3824 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03

RIC: 564.3796

Chemical Formula: $C_{34}H_{50}N_3O_4^+$
Exact Mass: 564.3796

NL: 1.23E5
m/z= 567.3956-567.4012 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03

RIC: 567.3984

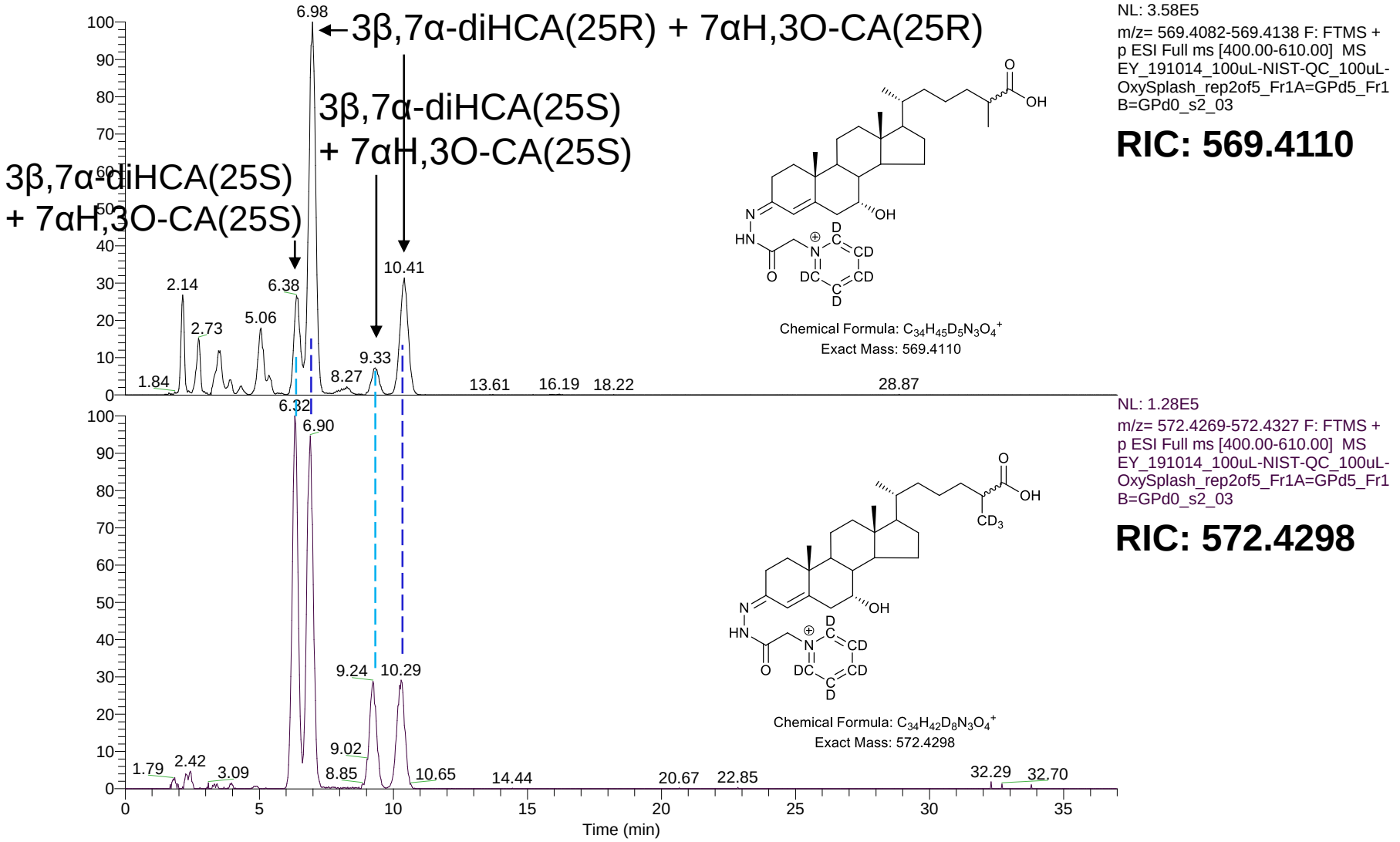
Chemical Formula: $C_{34}H_{47}D_3N_3O_4^+$
Exact Mass: 567.3984

S6D

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS

10/15/19 02:44:12
ɹg Chol-D7(certified)

RT: 0.00 - 37.01



NL: 3.58E5
m/z= 569.4082-569.4138 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03

RIC: 569.4110

Chemical Formula: $C_{34}H_{45}D_5N_3O_4^+$
Exact Mass: 569.4110

NL: 1.28E5
m/z= 572.4269-572.4327 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_03

RIC: 572.4298

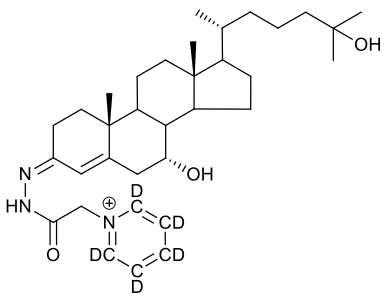
Chemical Formula: $C_{34}H_{42}D_8N_3O_4^+$
Exact Mass: 572.4298

S7A

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 555.43@cid30.00 471.36@cid35.00 [125.00-560.0

5.03 AV: 1 NL: 2.77E3

MS^3: 555→471→
5.03 min (17 min gradient)
7α,25-diHC + 7α,25-diHCO

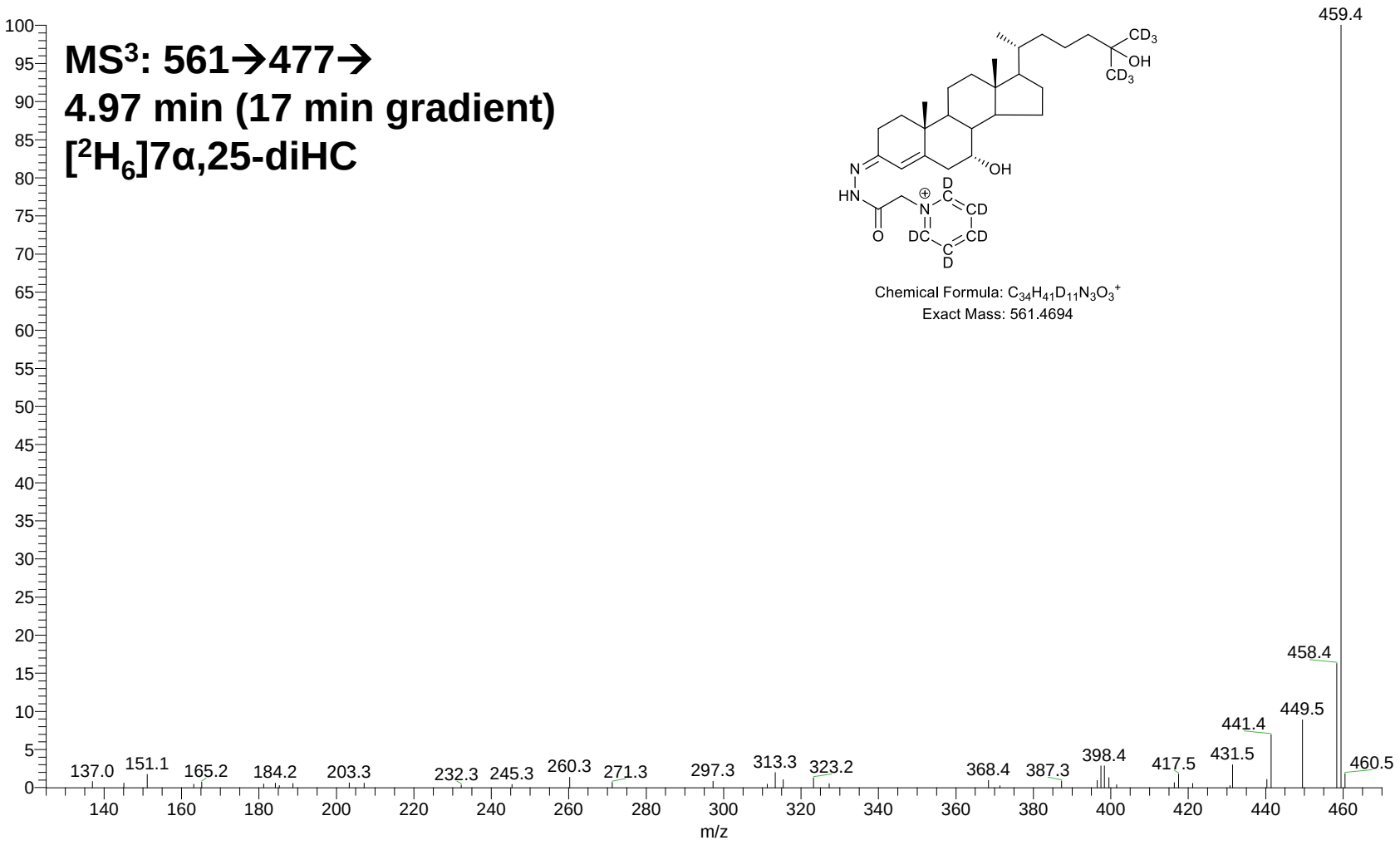


Chemical Formula: $C_{34}H_{47}D_5N_3O_3^+$
Exact Mass: 555.4317

S7B

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 561.47@cid30.00 477.40@cid35.00 [130.00-570.0

4.97 AV: 1 NL: 1.29E3

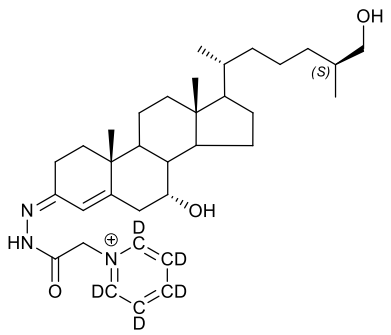


S7C

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 555.43@cid30.00 471.36@cid35.00 [125.00-560.0

5.43 AV: 1 NL: 5.27E2

MS^3: 555→471→
5.43 min (17 min gradient)
7α,(25S)26-diHC + 7α,(25S)26-diHCO



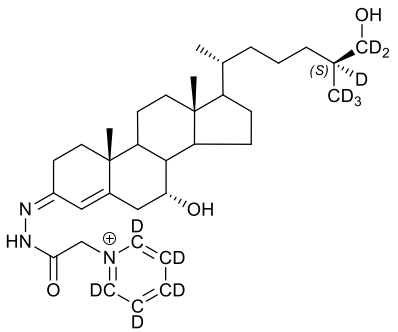
Chemical Formula: $C_{34}H_{47}D_5N_3O_3^+$
Exact Mass: 555.4317

S7D

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 561.47@cid30.00 477.40@cid35.00 [130.00-570.0

5.39 AV: 1 NL: 3.99E2

MS^3: 561→477→
5.39 min (17 min gradient)
[$^2H_6$]7α,(25S)26-diHC



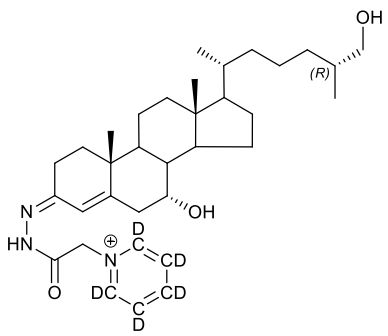
Chemical Formula: $C_{34}H_{41}D_{11}N_3O_3^+$
Exact Mass: 561.4694

S7E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 555.43@cid30.00 471.36@cid35.00 [125.00-560.0

5.64 AV: 1 NL: 3.64E3

MS^3: 555→471→
5.64 min (17 min gradient)
7α,(25R)26-diHC + 7α,(25R)26-diHCO

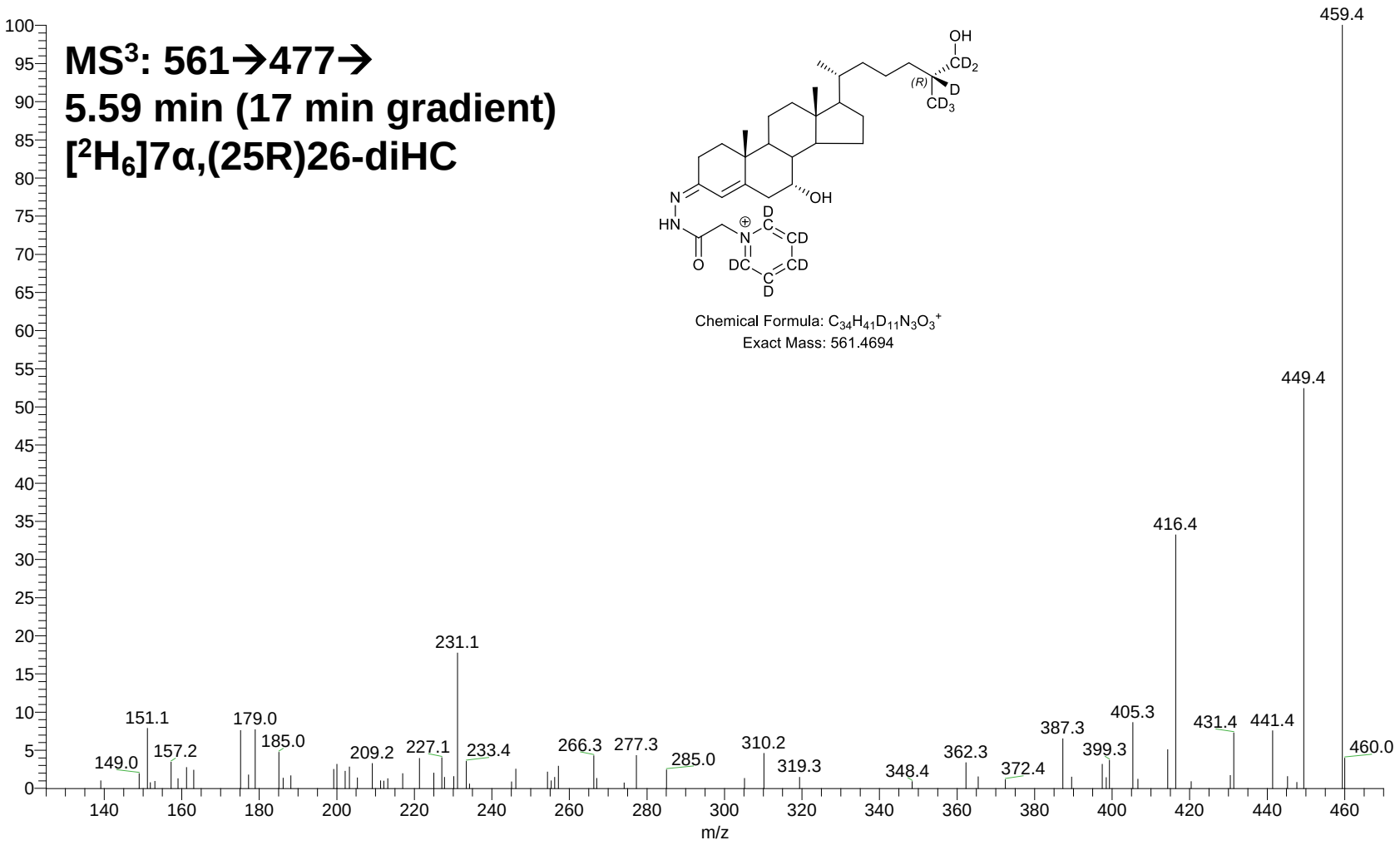


Chemical Formula: $C_{34}H_{47}D_5N_3O_3^+$
Exact Mass: 555.4317

S7F

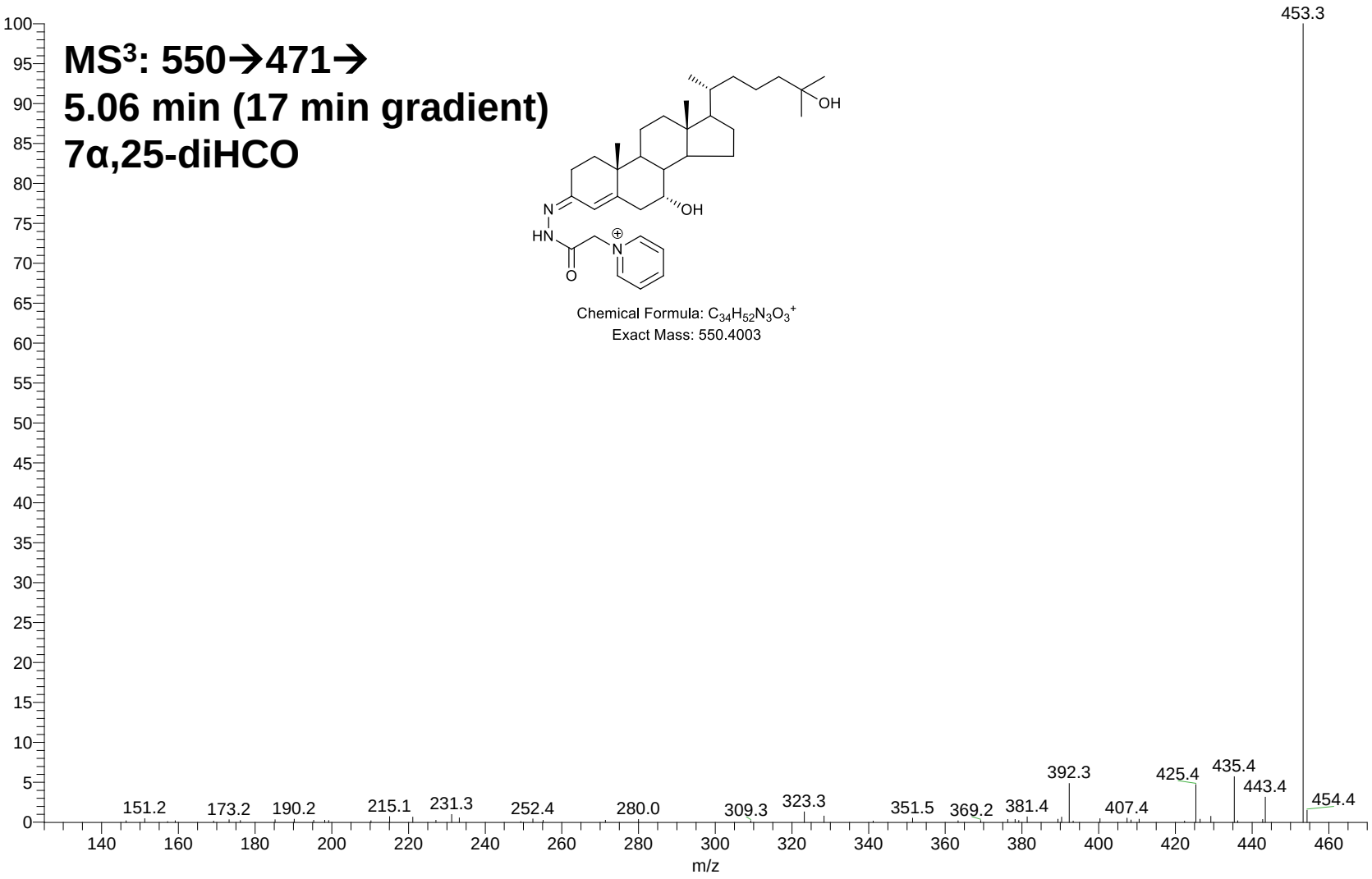
EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 561.47@cid30.00 477.40@cid35.00 [130.00-570.0

5.59 AV: 1 NL: 4.27E2



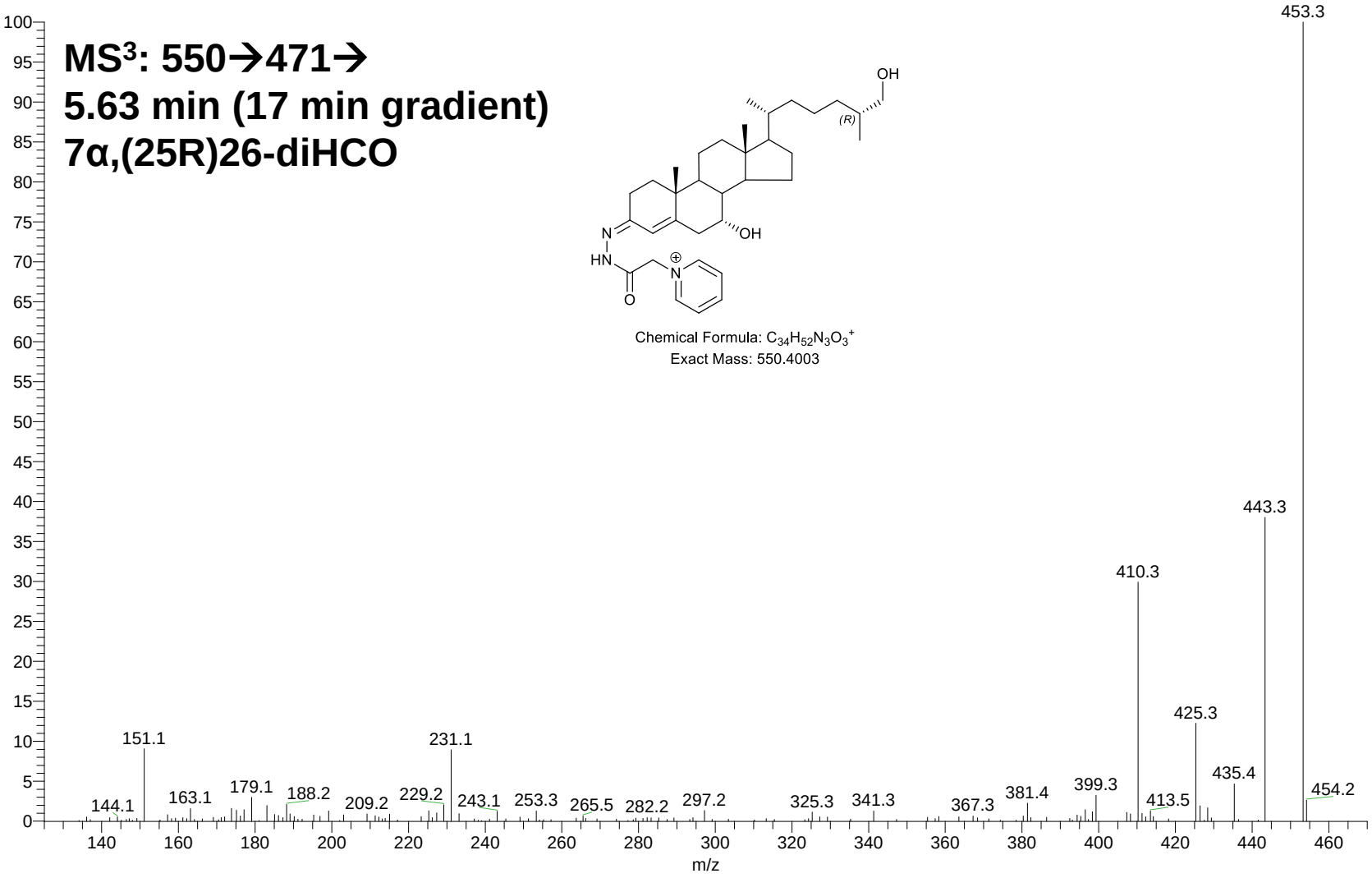
S7G

EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_04 #1078 RT: 5.06 AV: 1 NL: 2.88E3
F: ITMS + c ESI Full ms3 550.40@cid30.00 471.36@cid35.00 [125.00-56



S7H

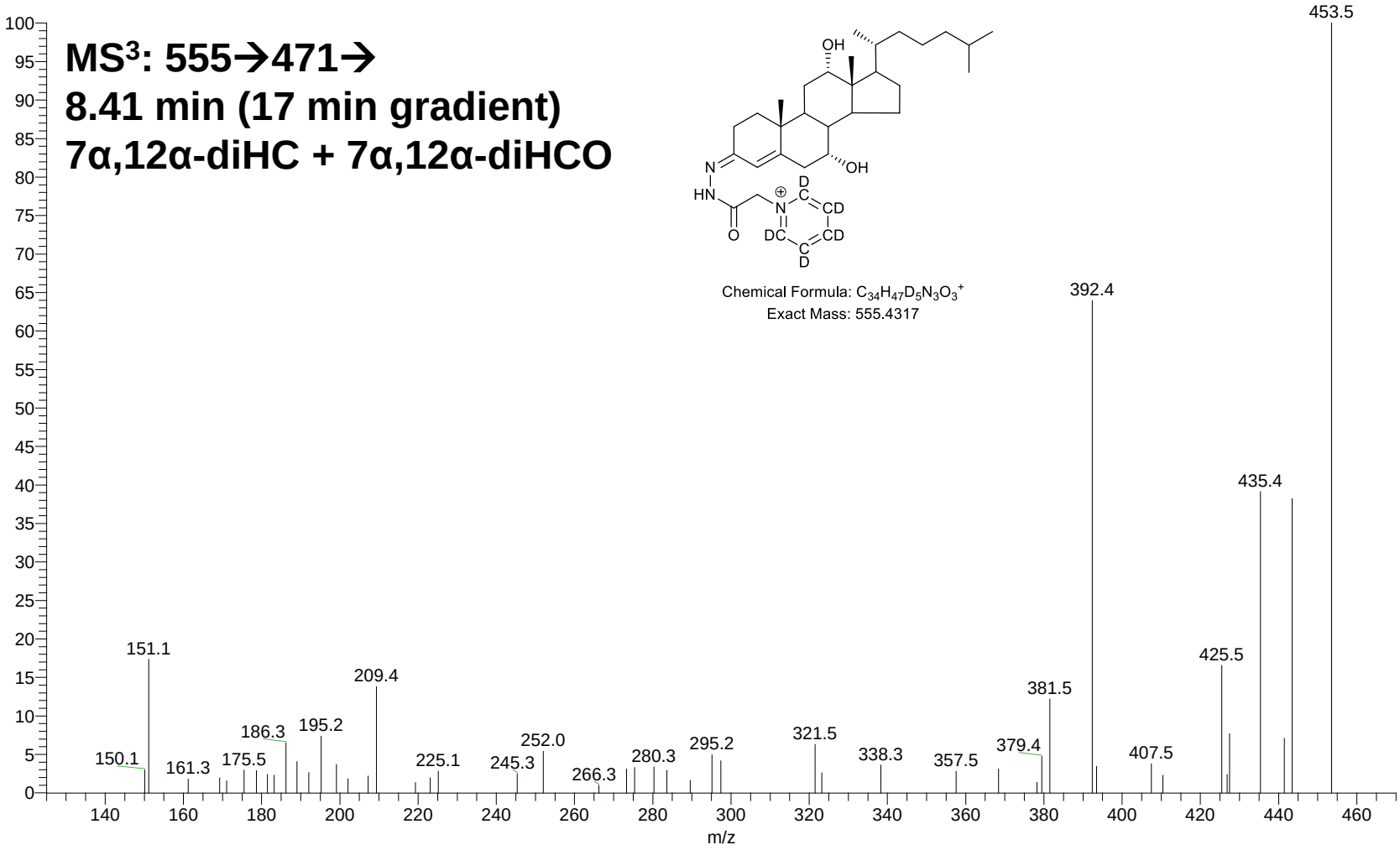
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_04 #1202 RT: 5.63 AV: 1 NL: 3.56E3
F: ITMS + c ESI Full ms3 550.40@cid30.00 471.36@cid35.00 [125.00-56



S7I

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:26:25
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 555.43@cid30.00 471.36@cid35.00 [125.00-560.0

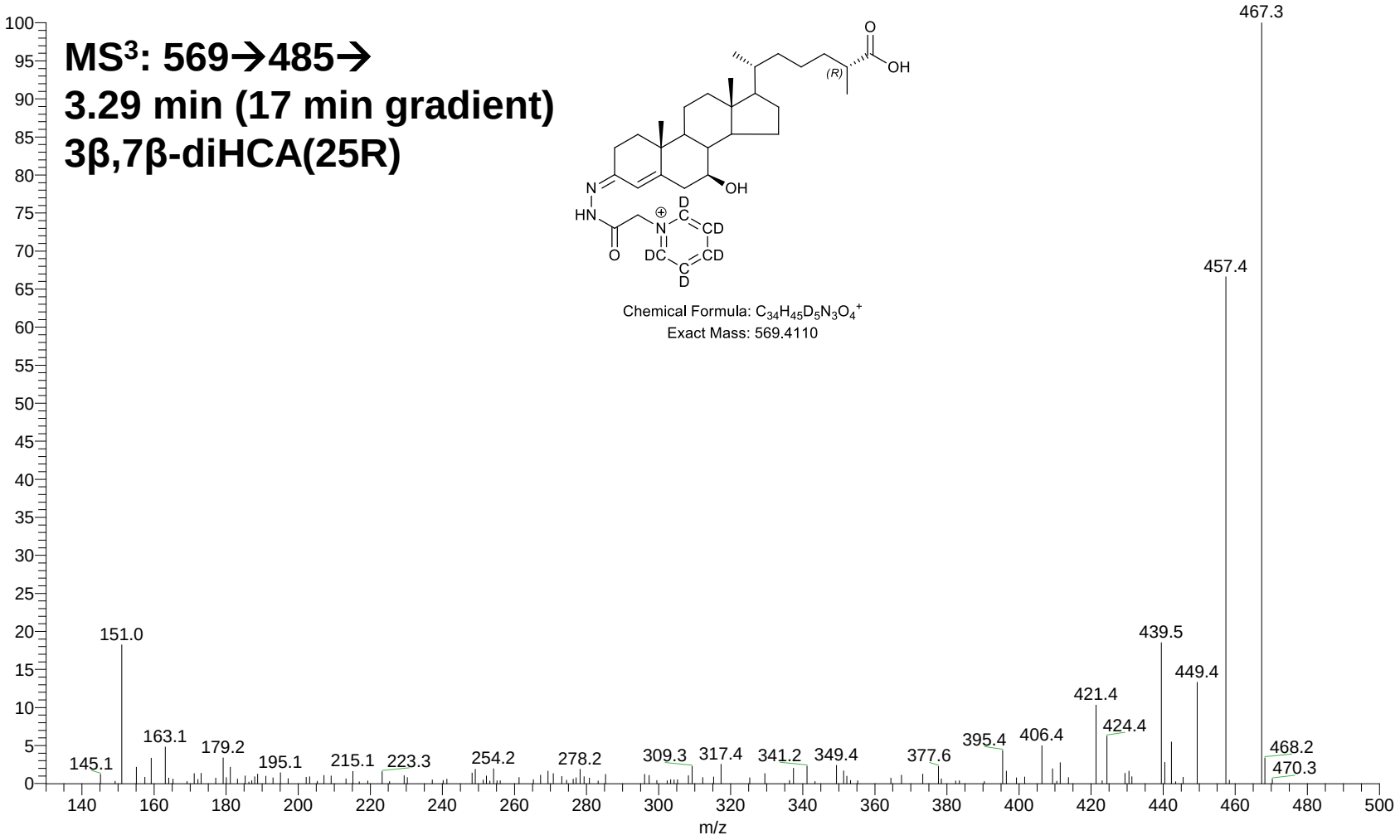
8.41 AV: 1 NL: 2.53E2



S8A

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

.29 AV: 1 NL: 1.23E3

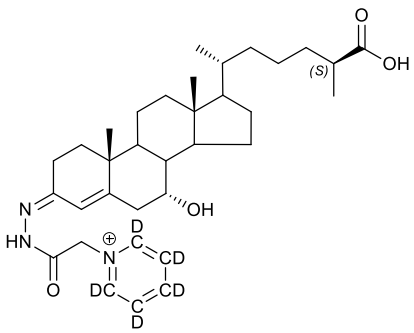


S8B

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

5.13 AV: 1 NL: 7.92E3

MS^3: 569→485→
5.13 min (17 min gradient)
3β,7α-diHCA(25S) + 7αH,3O-CA(25S)

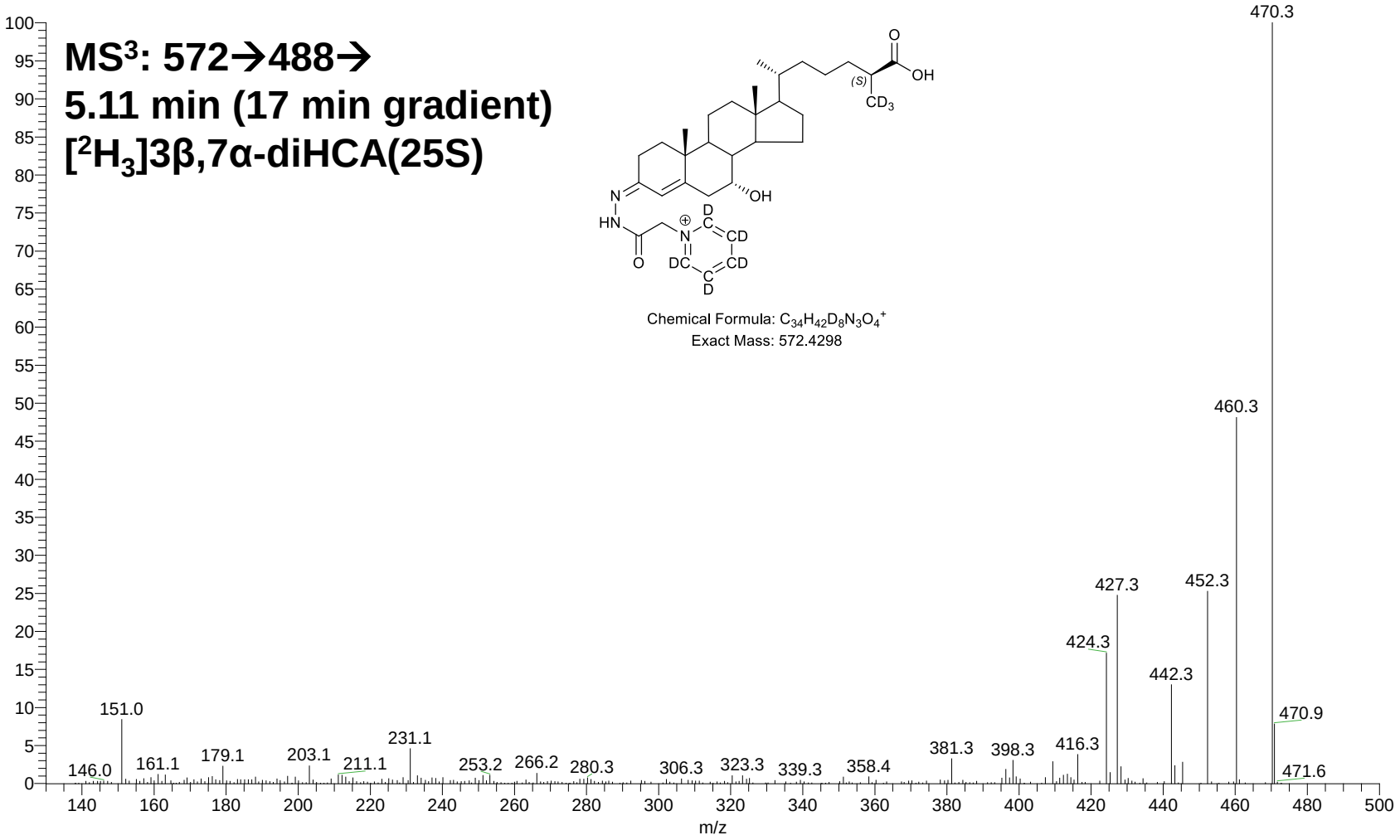


Chemical Formula: $C_{34}H_{45}D_5N_3O_4^+$
Exact Mass: 569.4110

S8C

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 572.43@cid30.00 488.36@cid35.00 [130.00-580.0

5.11 AV: 1 NL: 9.87E3

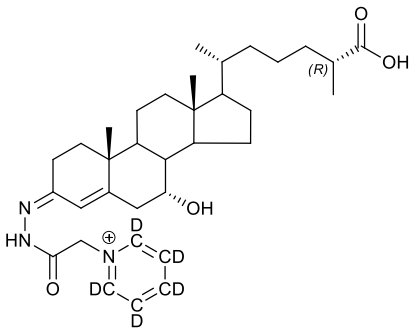


S8D

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

5.36 AV: 1 NL: 3.45E4

MS^3: 569→485→
5.36 min (17 min gradient)
3β,7α-diHCA(25R) + 7αH,3O-CA(25R)

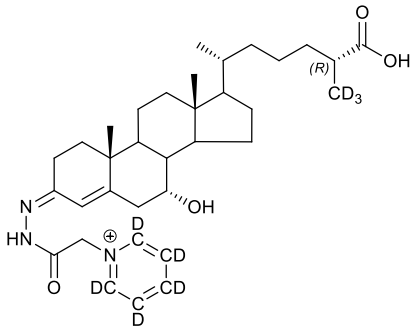


Chemical Formula: $C_{34}H_{45}D_5N_3O_4^+$
Exact Mass: 569.4110

S8E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E 5.30 AV: 1 NL: 1.20E4
F: ITMS + c ESI Full ms3 572.43@cid30.00 488.36@cid35.00 [130.00-580.0

MS^3: 572→488→
5.30 min (17 min gradient)
$[^2H_3]$3β,7α-diHCA(25R)



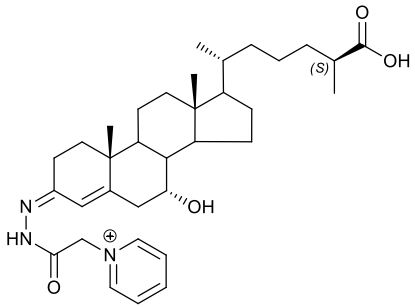
Chemical Formula: $C_{34}H_{42}D_8N_3O_4^+$
Exact Mass: 572.4298

S8F

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 564.38@cid30.00 485.34@cid35.00 [130.00-575.0

5.12 AV: 1 NL: 4.86E3

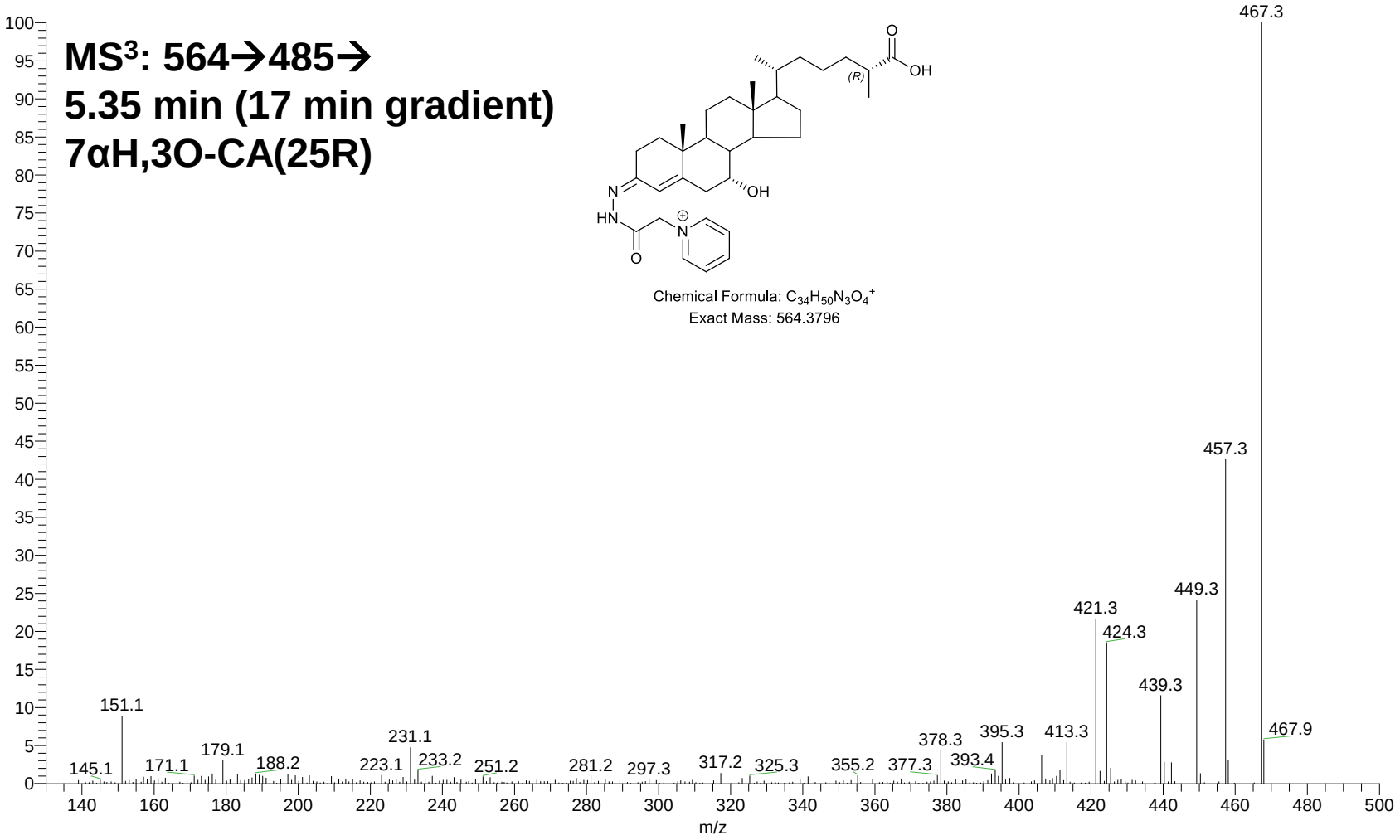
MS^3: 564→485→
5.12 min (17 min gradient)
7αH,3O-CA(25S)



Chemical Formula: $C_{34}H_{50}N_3O_4^+$
Exact Mass: 564.3796

S8G

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E 5.35 AV: 1 NL: 2.29E4
F: ITMS + c ESI Full ms3 564.38@cid30.00 485.34@cid35.00 [130.00-575.0

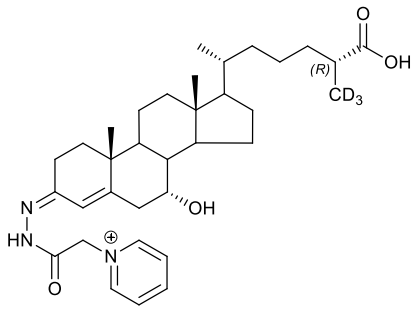


S8H

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 567.40@cid30.00 488.36@cid35.00 [130.00-580.0

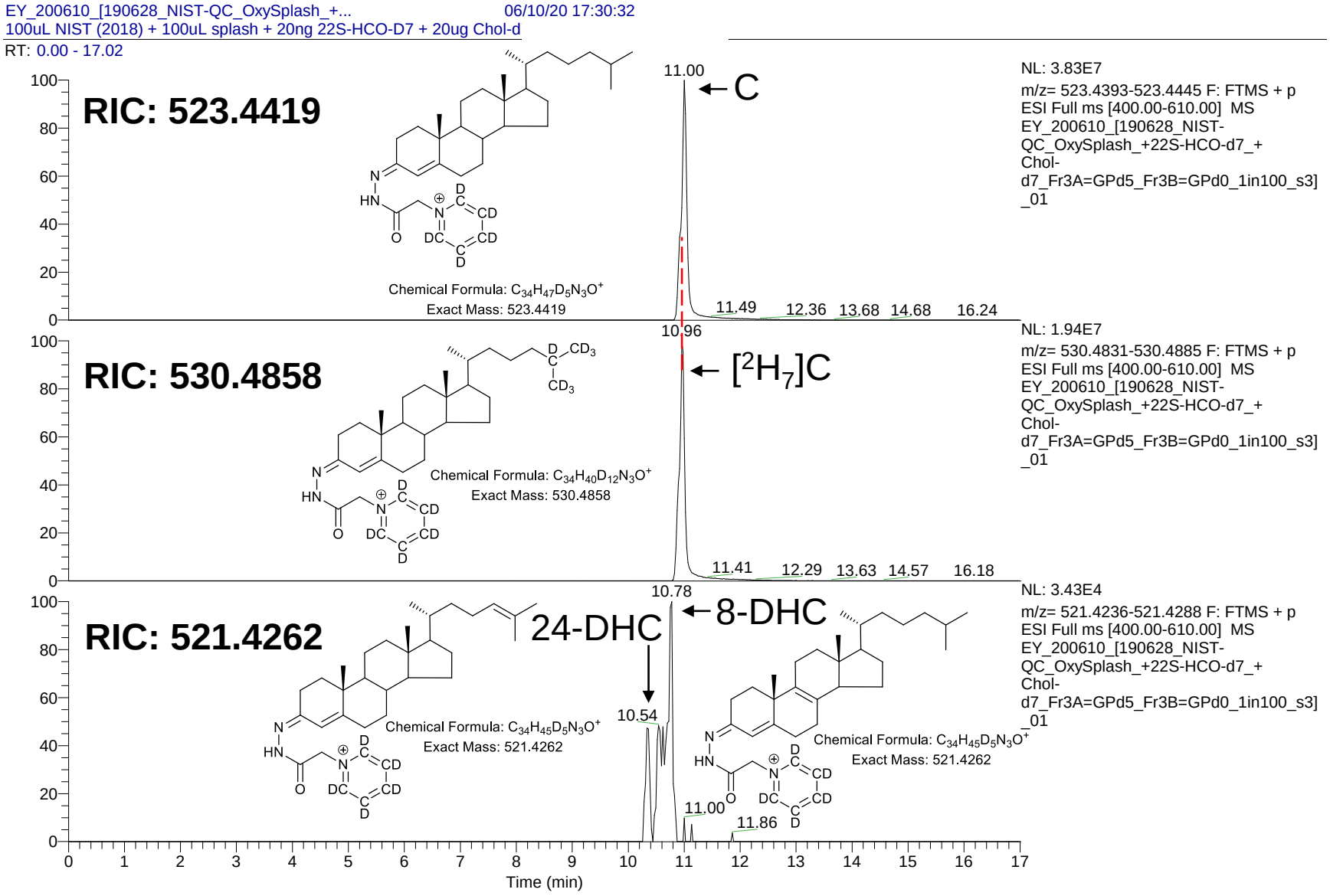
5.30 AV: 1 NL: 1.21E4

MS^3: 567→488→
5.30 min (17 min gradient)
[$^2H_3$]7αH,3O-CA(25R)



Chemical Formula: $C_{34}H_{47}D_3N_3O_4^+$
Exact Mass: 567.3984

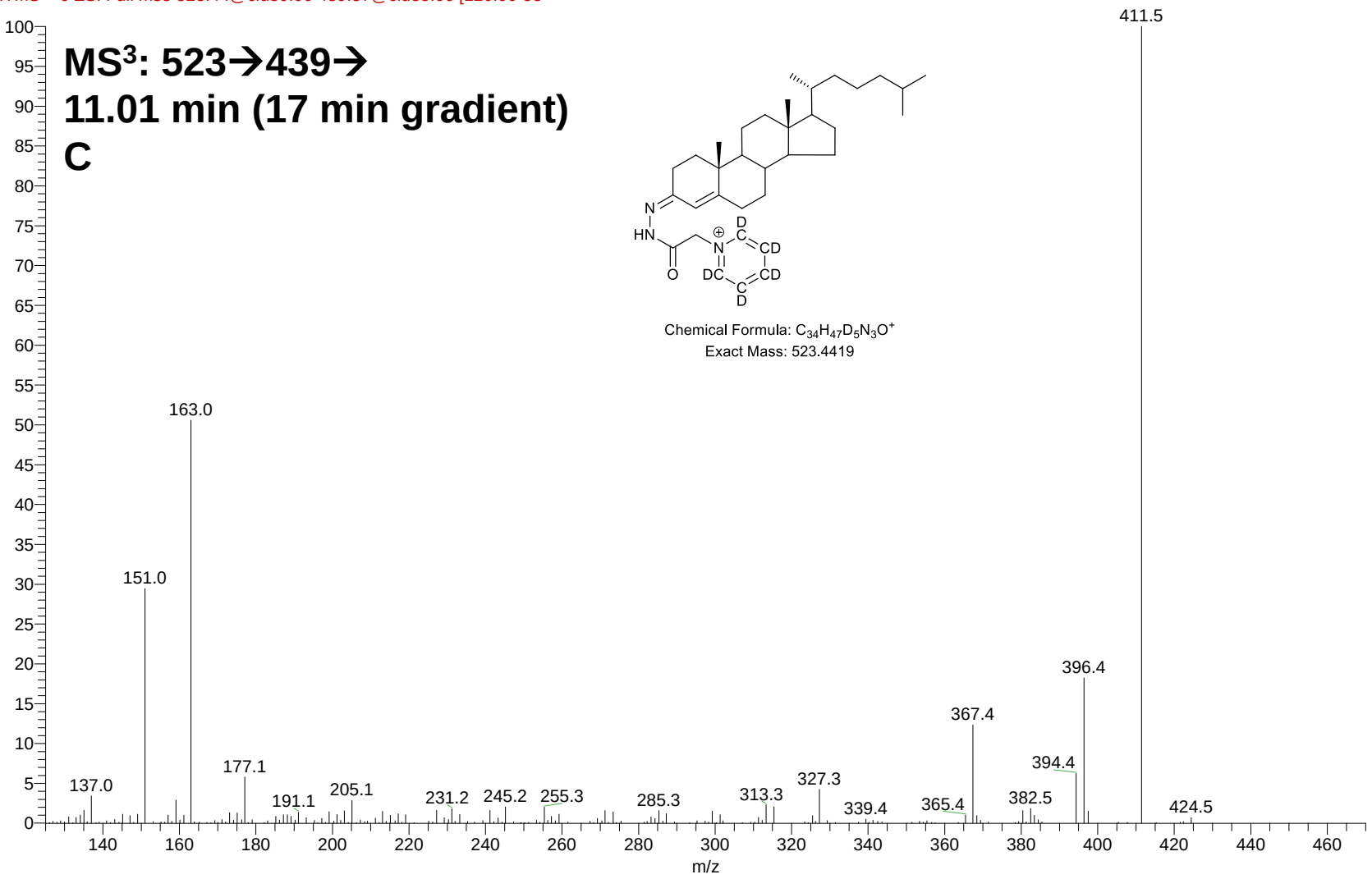
S9A


EY_200610_[190628_NIST-QC_OxySplash_+...
06/10/20 17:30:32
100uL NIST (2018) + 100uL splash + 20ng 22S-HCO-D7 + 20ug Chol-d
RT: 0.00 - 17.02
RIC: 523.4419
11.00
C
Chemical Formula: C34H47D5N3O+
Exact Mass: 523.4419
11.49
12.36
13.68
14.68
16.24
NL: 3.83E7
m/z= 523.4393-523.4445 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr3A=GPd5_Fr3B=GPd0_1in100_s3]_01
RIC: 530.4858
10.96
[2H7]C
Chemical Formula: C34H40D12N3O+
Exact Mass: 530.4858
11.41
12.29
13.63
14.57
16.18
NL: 1.94E7
m/z= 530.4831-530.4885 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr3A=GPd5_Fr3B=GPd0_1in100_s3]_01
RIC: 521.4262
10.78
8-DHC
24-DHC
10.54
Chemical Formula: C34H45D5N3O+
Exact Mass: 521.4262
Chemical Formula: C34H45D5N3O+
Exact Mass: 521.4262
11.00
11.86
NL: 3.43E4
m/z= 521.4236-521.4288 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr3A=GPd5_Fr3B=GPd0_1in100_s3]_01
Time (min)

S9B

EY_200610_[190628_NIST-QC_OxySplash_+... 06/10/20 17:30:32
100uL NIST (2018) + 100uL splash + 20ng 22S-HCO-D7 + 20ug Chol-d7
EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr
F: ITMS + c ESI Full ms3 523.44@cid30.00 439.37@cid35.00 [120.00-53

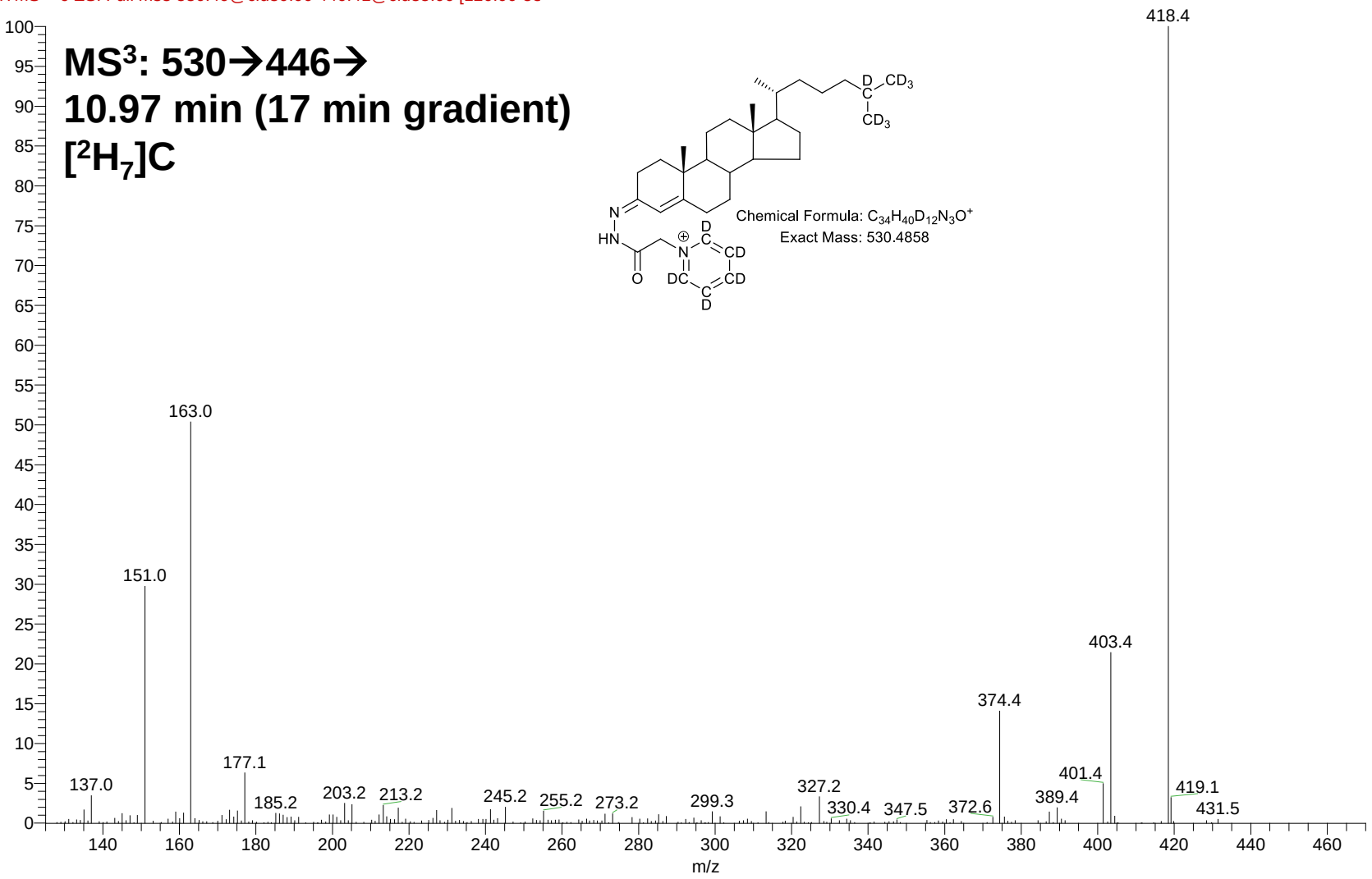
00_s3]_01 #2379 RT: 11.01 AV: 1 NL: 2.73E6



S9C

EY_200610_[190628_NIST-QC_OxySplash_+... 06/10/20 17:30:32
100uL NIST (2018) + 100uL splash + 20ng 22S-HCO-D7 + 20ug Chol-d7
EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr
F: ITMS + c ESI Full ms3 530.49@cid30.00 446.41@cid35.00 [120.00-53

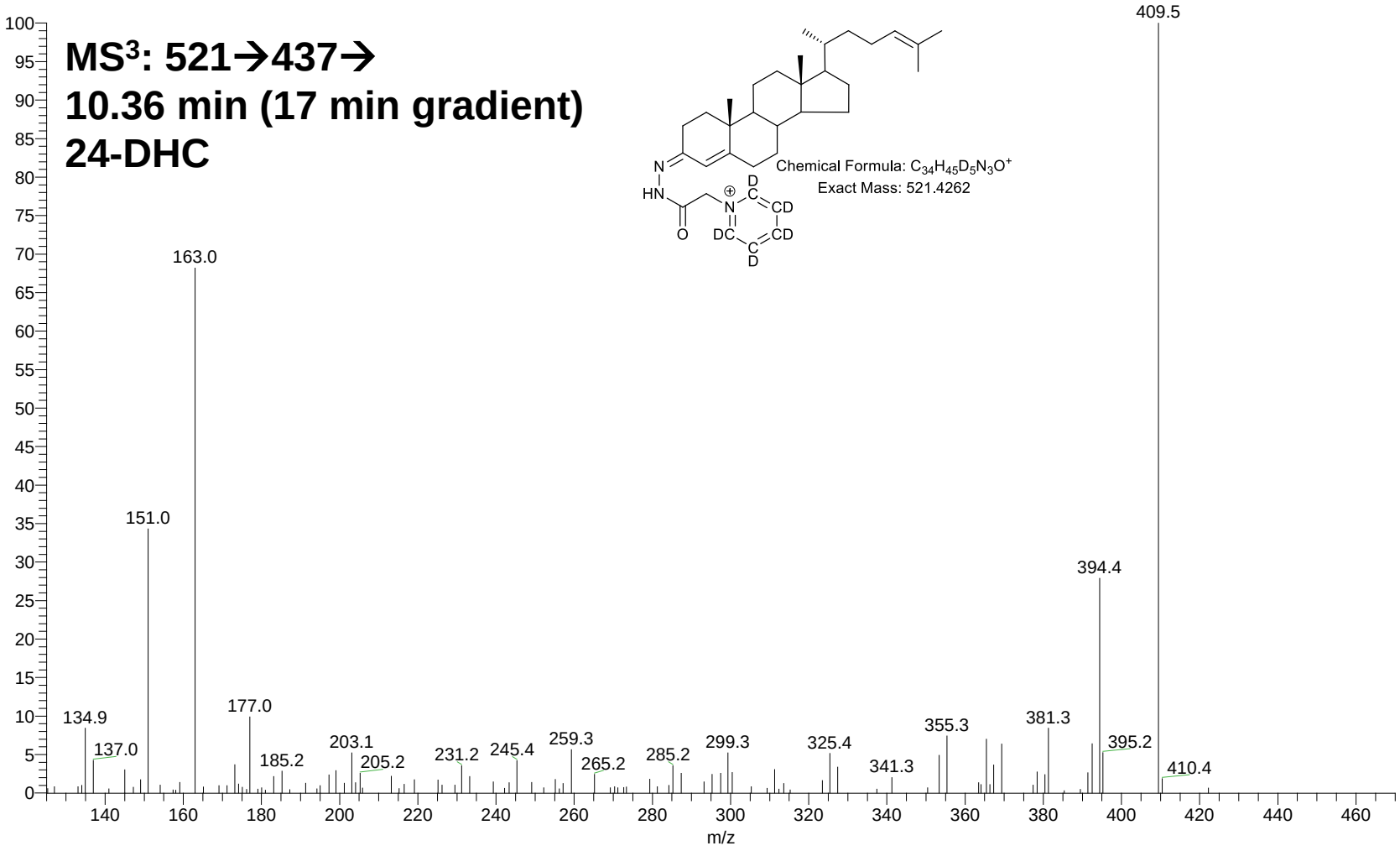
00_s3]_01 #2368 RT: 10.97 AV: 1 NL: 1.37E6



S9D

EY_200610_[190628_NIST-QC_OxySplash_+... 06/10/20 17:50:25
100uL NIST (2018) + 100uL splash + 20ng 22S-HCO-D7 + 20ug Chol-d7 (c
EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr3A
F: ITMS + c ESI Full ms3 521.43@cid30.00 437.35@cid35.00 [120.00-530.(

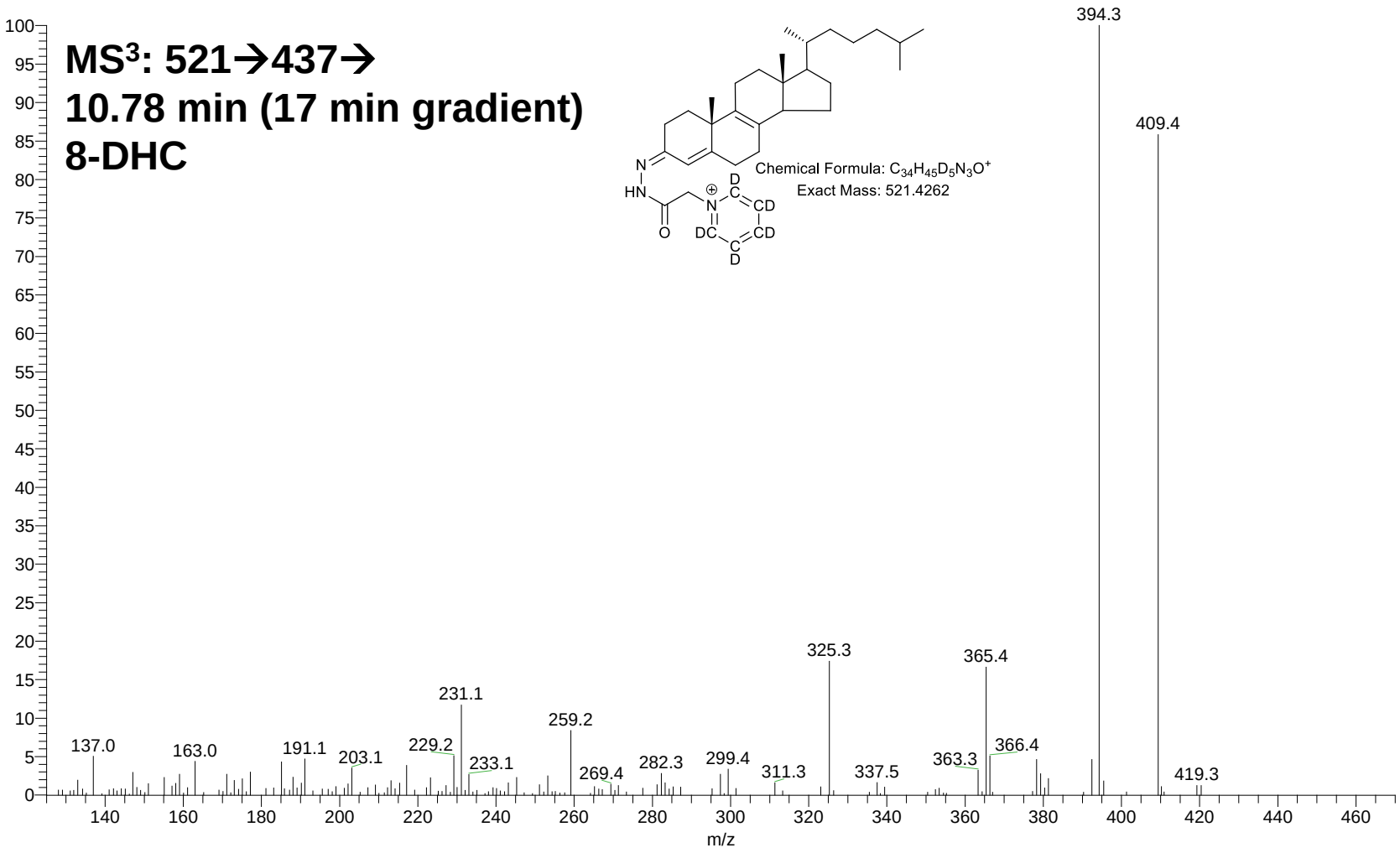
_s3]_02 #2232 RT: 10.36 AV: 1 NL: 1.14E3



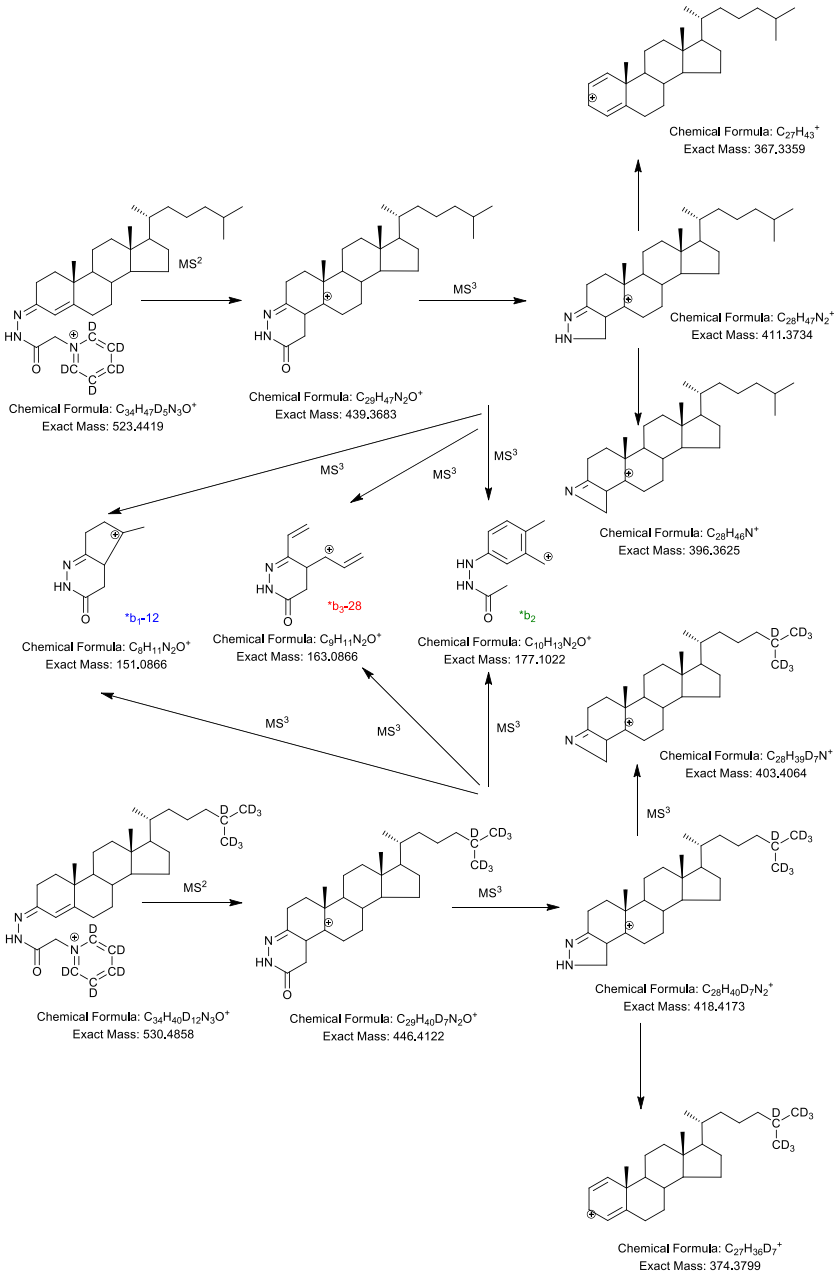
S9E

EY_200610_[190628_NIST-QC_OxySplash_+... 06/10/20 17:50:25
100uL NIST (2018) + 100uL splash + 20ng 22S-HCO-D7 + 20ug Chol-d7 (c
EY_200610_[190628_NIST-QC_OxySplash_+22S-HCO-d7_+Chol-d7_Fr3A
F: ITMS + c ESI Full ms3 521.43@cid30.00 437.35@cid35.00 [120.00-530.(

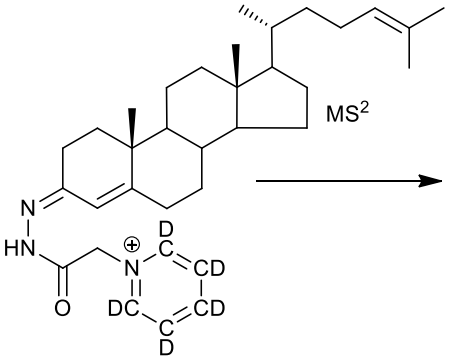
_s3]_02 #2328 RT: 10.76 AV: 1 NL: 2.80E3



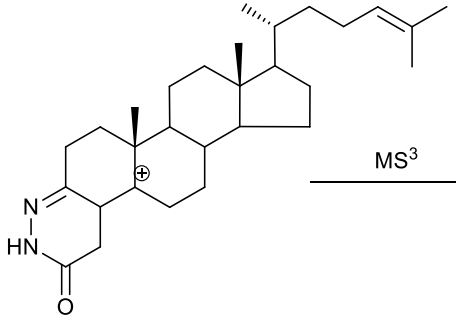
S9F


MS2
MS3
MS3
MS3
MS3
MS3
MS3
MS3
MS3
MS2
MS3
Chemical Formula: C34H47D5N3O+
Exact Mass: 523.4419
Chemical Formula: C29H47N2O+
Exact Mass: 439.3683
Chemical Formula: C27H43+
Exact Mass: 367.3359
Chemical Formula: C28H47N2+
Exact Mass: 411.3734
Chemical Formula: C28H46N+
Exact Mass: 396.3625
*b1-12
Chemical Formula: C8H11N2O+
Exact Mass: 151.0866
*b3-28
Chemical Formula: C8H11N2O+
Exact Mass: 163.0866
*b2
Chemical Formula: C10H13N2O+
Exact Mass: 177.1022
Chemical Formula: C28H39D7N+
Exact Mass: 403.4064
Chemical Formula: C34H40D12N3O+
Exact Mass: 530.4858
Chemical Formula: C29H40D7N2O+
Exact Mass: 446.4122
Chemical Formula: C28H40D7N2+
Exact Mass: 418.4173
Chemical Formula: C27H36D7+
Exact Mass: 374.3799

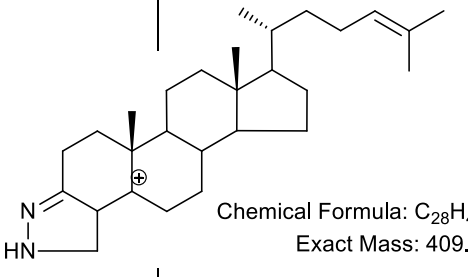
S9G



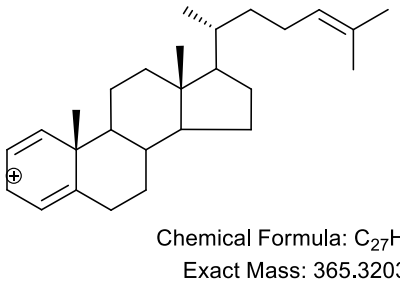
Chemical Formula: $C_{34}H_{45}D_5N_3O^+$
Exact Mass: 521.4262



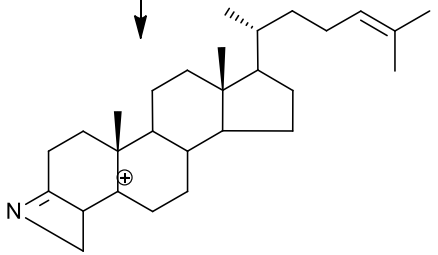
Chemical Formula: $C_{29}H_{45}N_2O^+$
Exact Mass: 437.3526



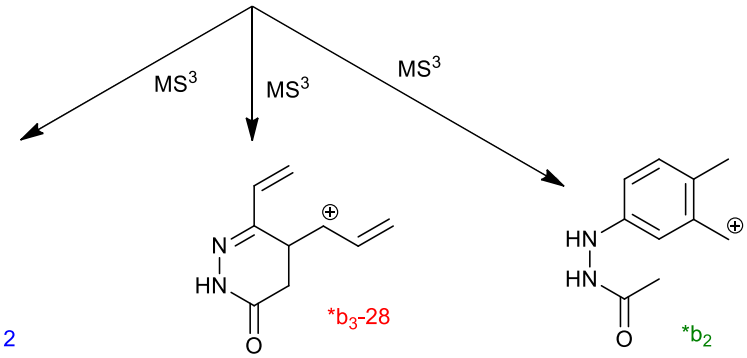
Chemical Formula: $C_{28}H_{45}N_2^+$
Exact Mass: 409.3577



Chemical Formula: $C_{27}H_{41}^+$
Exact Mass: 365.3203



Chemical Formula: $C_{28}H_{44}N^+$
Exact Mass: 394.3468

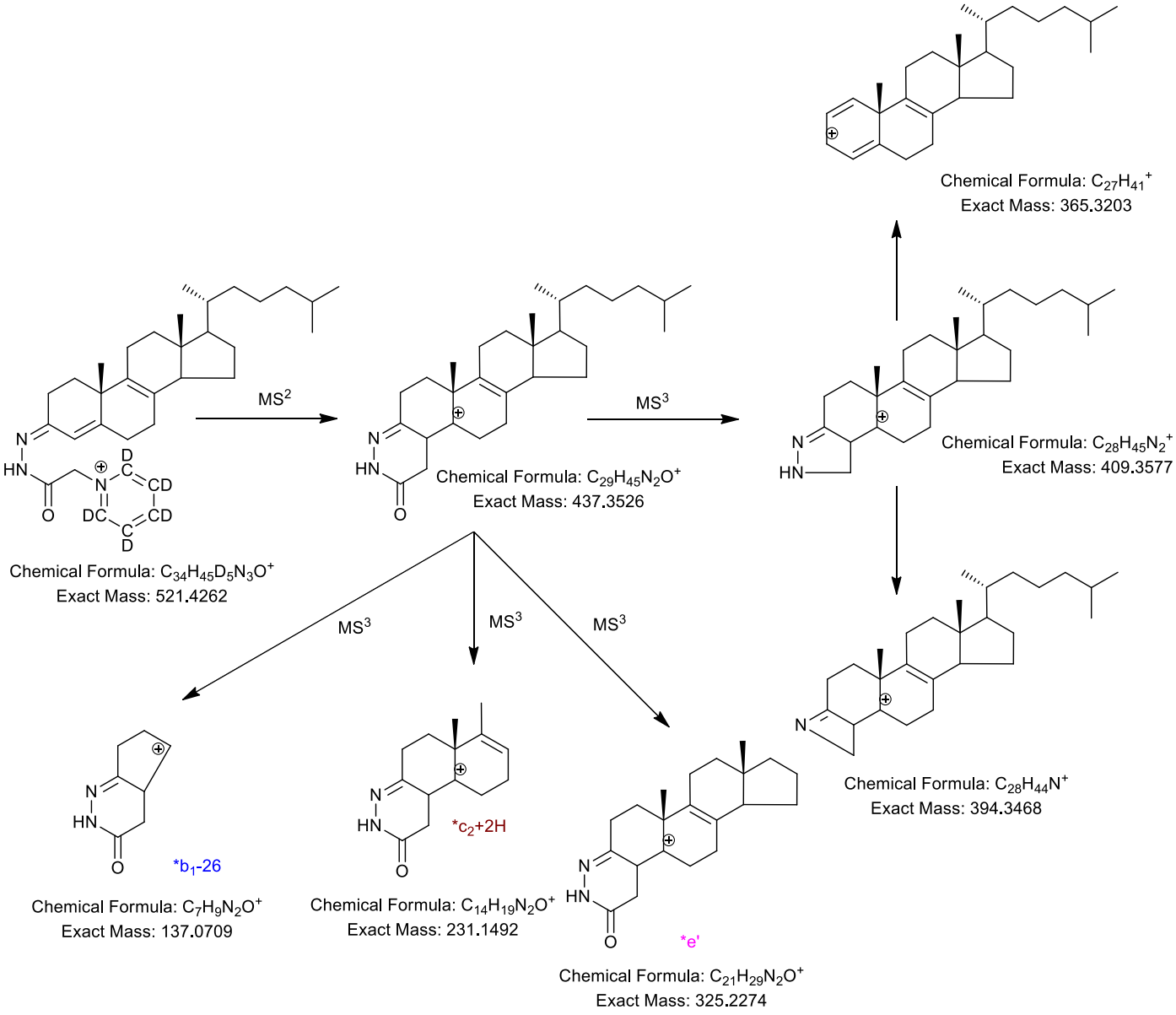


Chemical Formula: $C_8H_{11}N_2O^+$
Exact Mass: 151.0866

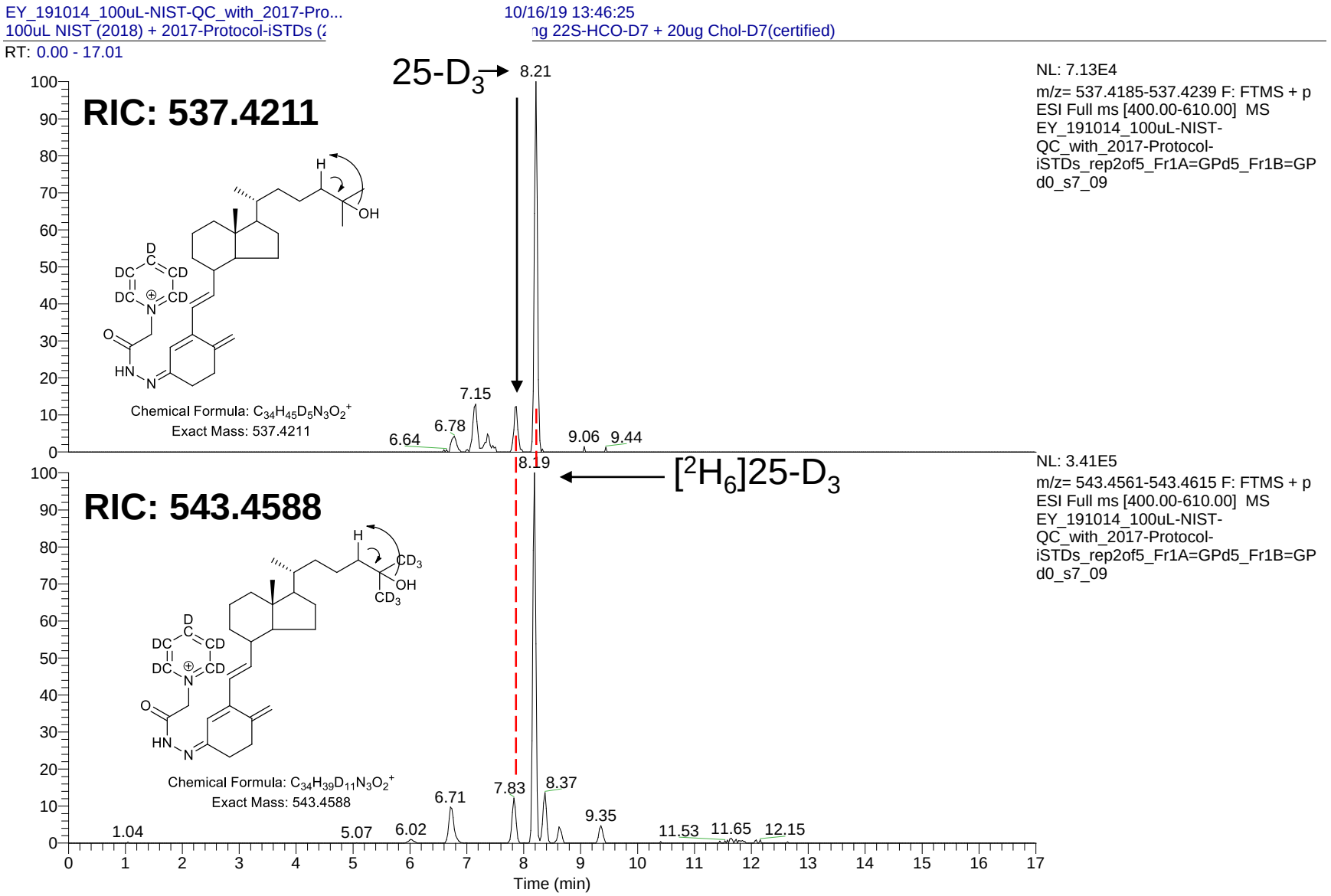
Chemical Formula: $C_9H_{11}N_2O^+$
Exact Mass: 163.0866

Chemical Formula: $C_{10}H_{13}N_2O^+$
Exact Mass: 177.1022

S9H

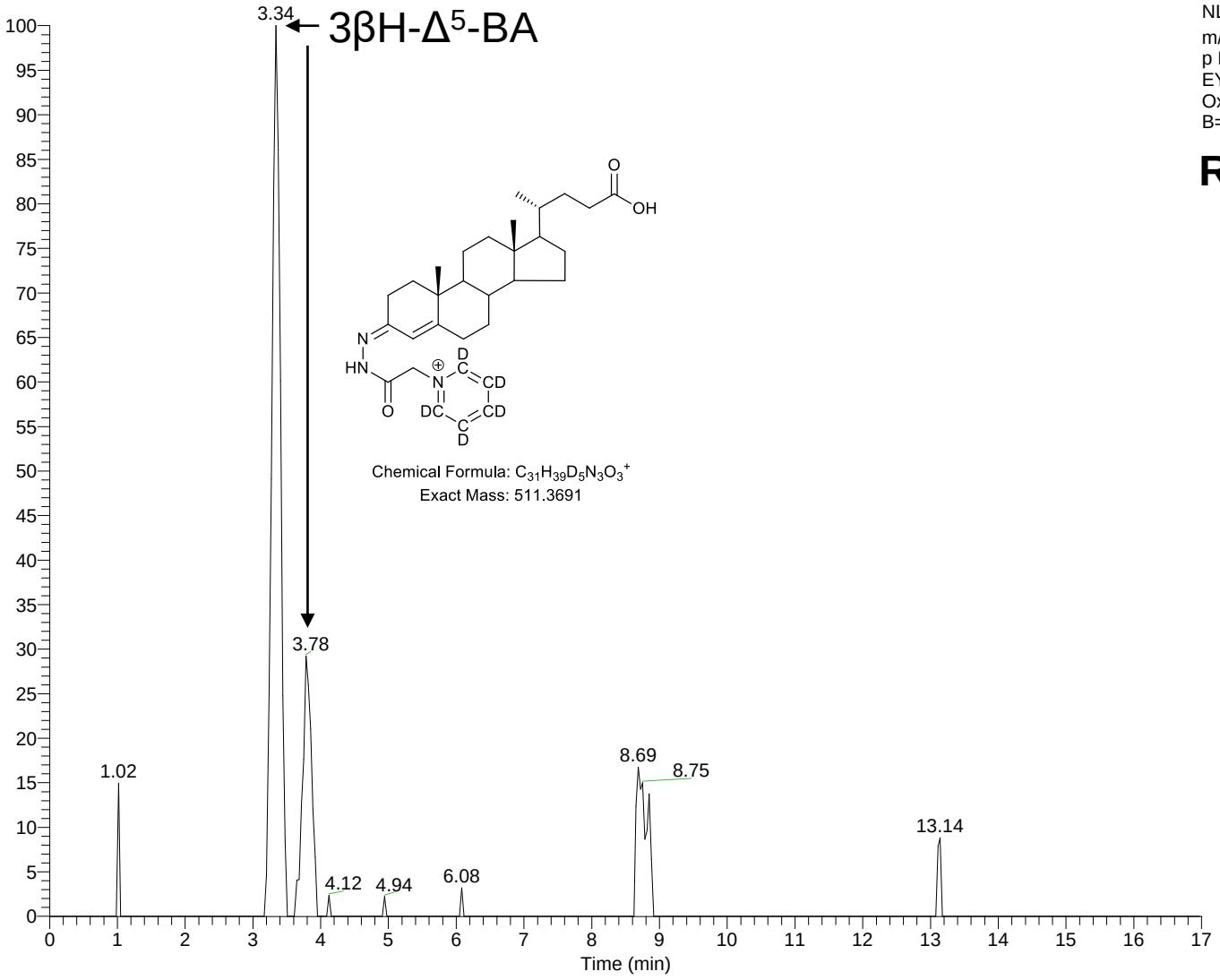

MS2
MS3
MS3
MS3
MS3
Chemical Formula: C34H45D5N3O+
Exact Mass: 521.4262
Chemical Formula: C29H45N2O+
Exact Mass: 437.3526
Chemical Formula: C28H45N2+
Exact Mass: 409.3577
Chemical Formula: C27H41+
Exact Mass: 365.3203
Chemical Formula: C28H44N+
Exact Mass: 394.3468
*b1-26
Chemical Formula: C7H9N2O+
Exact Mass: 137.0709
*c2+2H
Chemical Formula: C14H19N2O+
Exact Mass: 231.1492
*e'
Chemical Formula: C21H29N2O+
Exact Mass: 325.2274

S10A


EY_191014_100uL-NIST-QC_with_2017-Pro...
100uL NIST (2018) + 2017-Protocol-iSTDs (2
10/16/19 13:46:25
ng 22S-HCO-D7 + 20ug Chol-D7(certified)
RT: 0.00 - 17.01
25-D3
8.21
RIC: 537.4211
NL: 7.13E4
m/z= 537.4185-537.4239 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_with_2017-Protocol-iSTDs_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s7_09
Chemical Formula: C34H45D5N3O2+
Exact Mass: 537.4211
7.15
6.78
6.64
9.06
9.44
8.19
[2H6]25-D3
RIC: 543.4588
NL: 3.41E5
m/z= 543.4561-543.4615 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_with_2017-Protocol-iSTDs_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s7_09
Chemical Formula: C34H39D11N3O2+
Exact Mass: 543.4588
7.83
8.37
6.71
9.35
1.04
5.07
6.02
11.53
11.65
12.15
Time (min)

S10B

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 05:51:45
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 2(
RT: 0.00 - 17.01

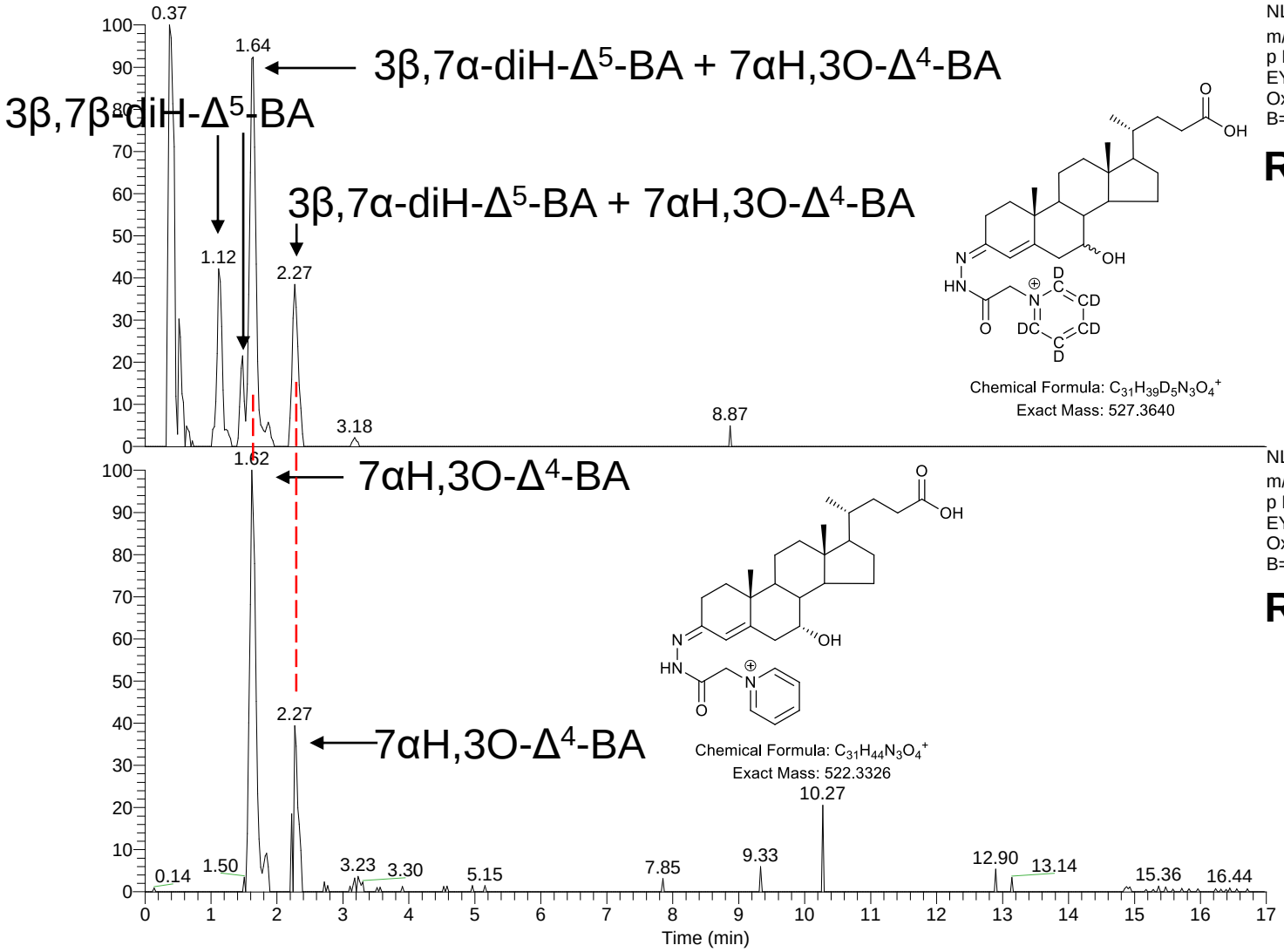


NL: 1.50E4
m/z= 511.3665-511.3717 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_11

RIC: 511.3691

S10C

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:07:57
100uL NIST (2018) + 100ul OxysterolSPLAS ug Chol-D7(certified)
RT: 0.00 - 17.00



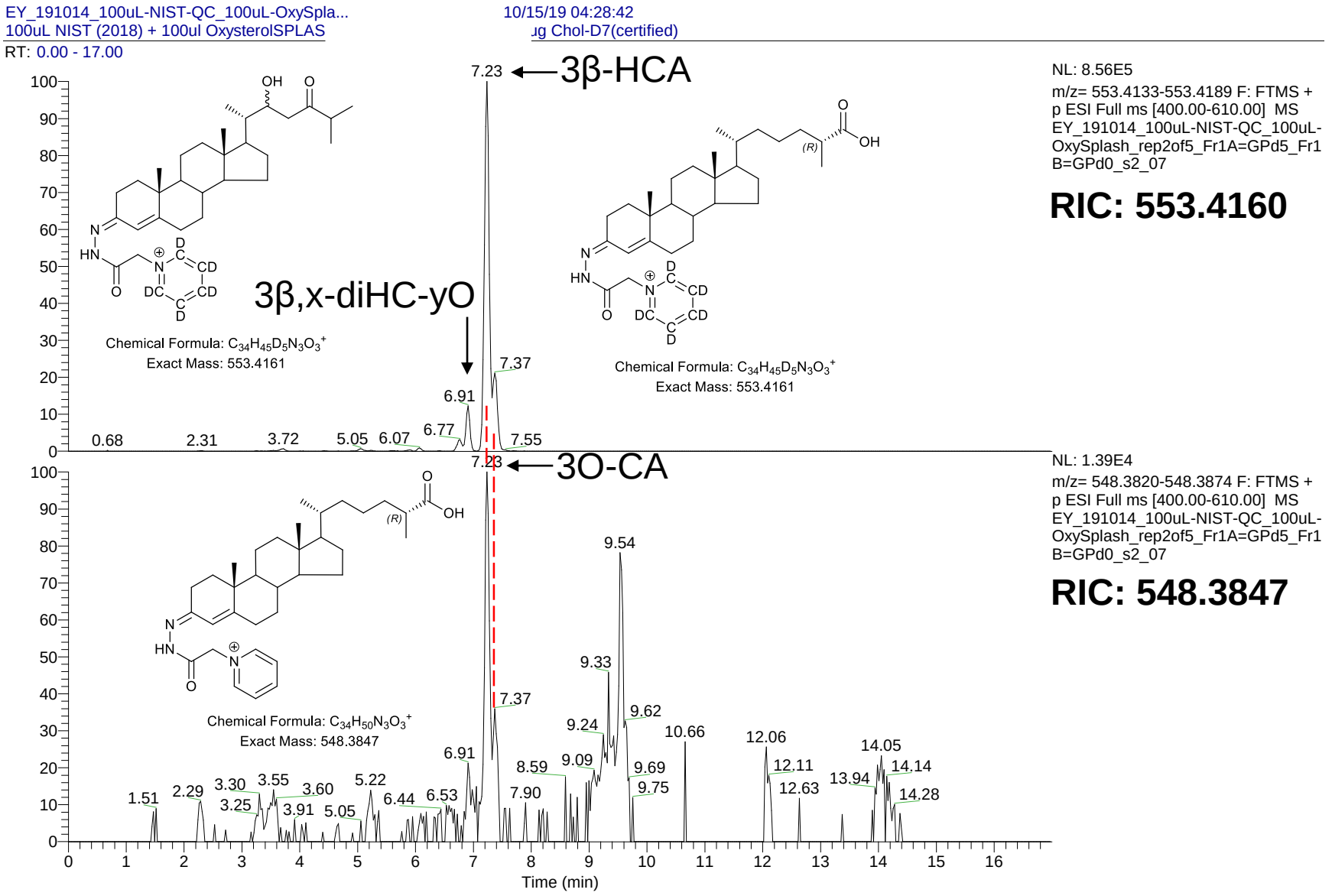
NL: 4.95E4
m/z= 527.3614-527.3666 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_06

RIC: 527.3640

NL: 2.67E4
m/z= 522.3300-522.3352 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_06

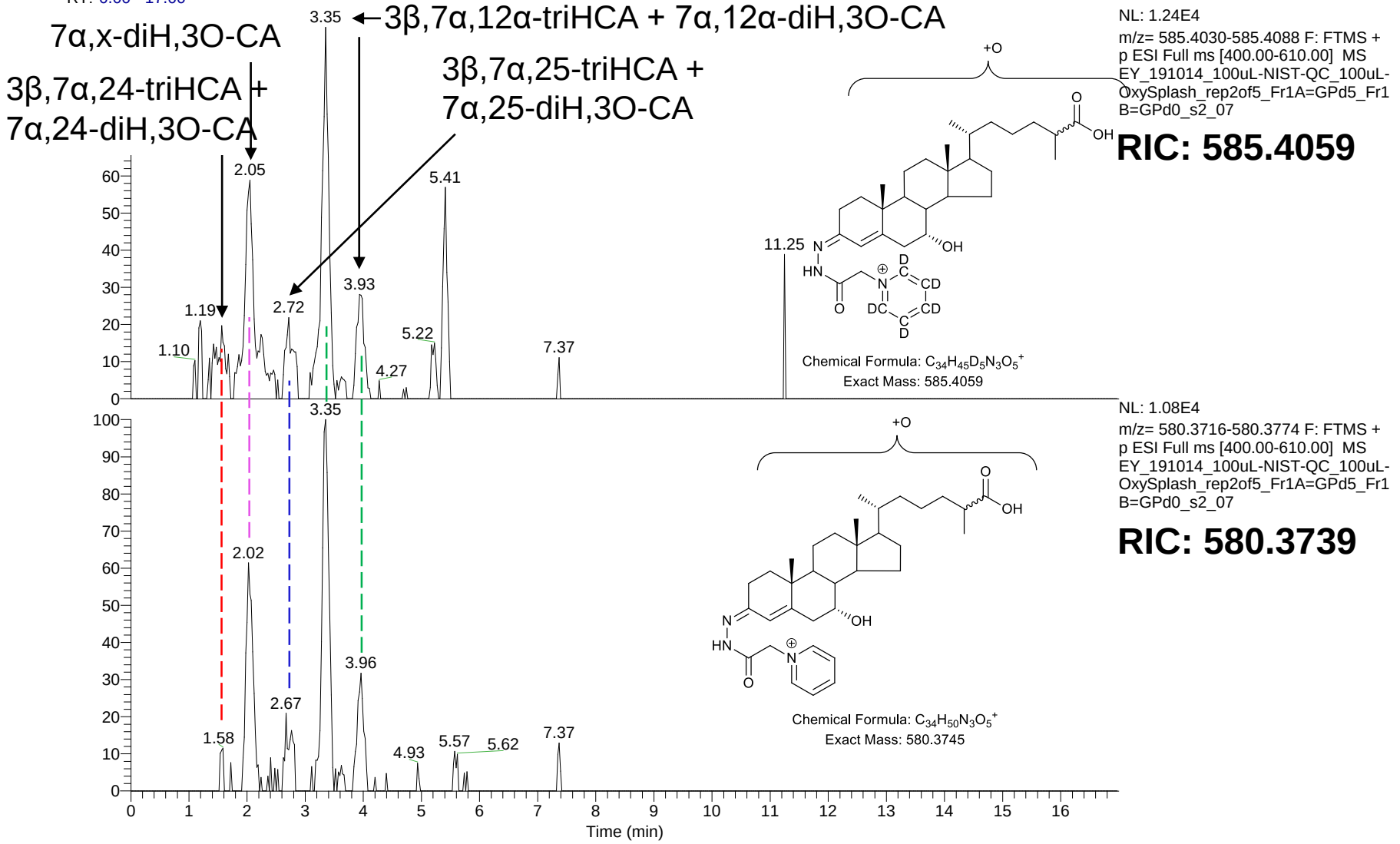
RIC: 522.3326

S10D


EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS
10/15/19 04:28:42
ug Chol-D7(certified)
RT: 0.00 - 17.00
7.23
3β-HCA
3β,x-diHC-yO
Chemical Formula: C34H45D5N3O3+
Exact Mass: 553.4161
Chemical Formula: C34H45D5N3O3+
Exact Mass: 553.4161
NL: 8.56E5
m/z= 553.4133-553.4189 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07
RIC: 553.4160
7.23
3O-CA
Chemical Formula: C34H50N3O3+
Exact Mass: 548.3847
NL: 1.39E4
m/z= 548.3820-548.3874 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07
RIC: 548.3847
Time (min)

S10E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLAS ug Chol-D7(certified)
RT: 0.00 - 17.00



Chemical Formula: $C_{34}H_{45}D_5N_3O_5^+$
Exact Mass: 585.4059

NL: 1.24E4
m/z= 585.4030-585.4088 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07

RIC: 585.4059

Chemical Formula: $C_{34}H_{50}N_3O_5^+$
Exact Mass: 580.3745

NL: 1.08E4
m/z= 580.3716-580.3774 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07

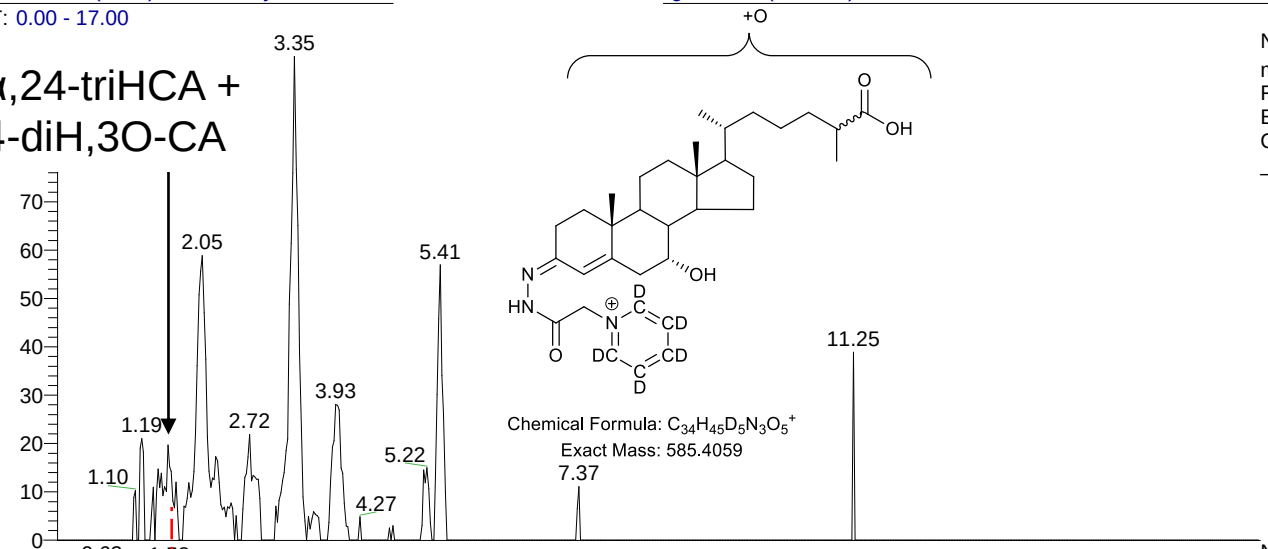
RIC: 580.3739

S10F

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS
RT: 0.00 - 17.00

10/15/19 04:28:42
ɹg Chol-D7(certified)

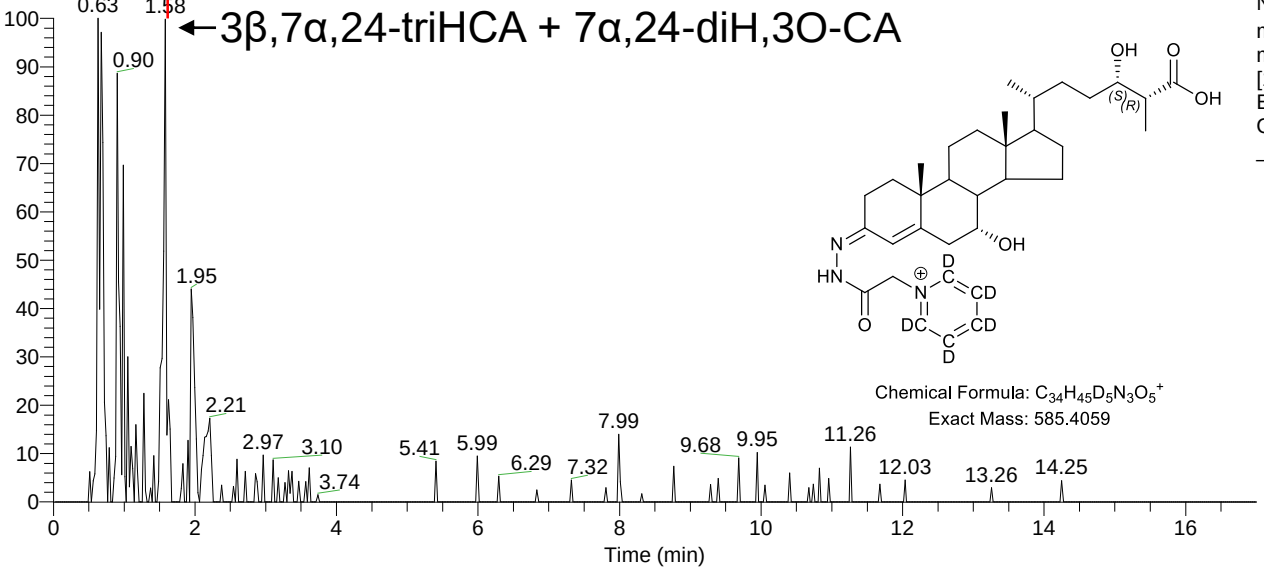
3β,7α,24-triHCA +
7α,24-diH,3O-CA



NL: 1.24E4
m/z= 585.4030-585.4088 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07

RIC: 585.4059

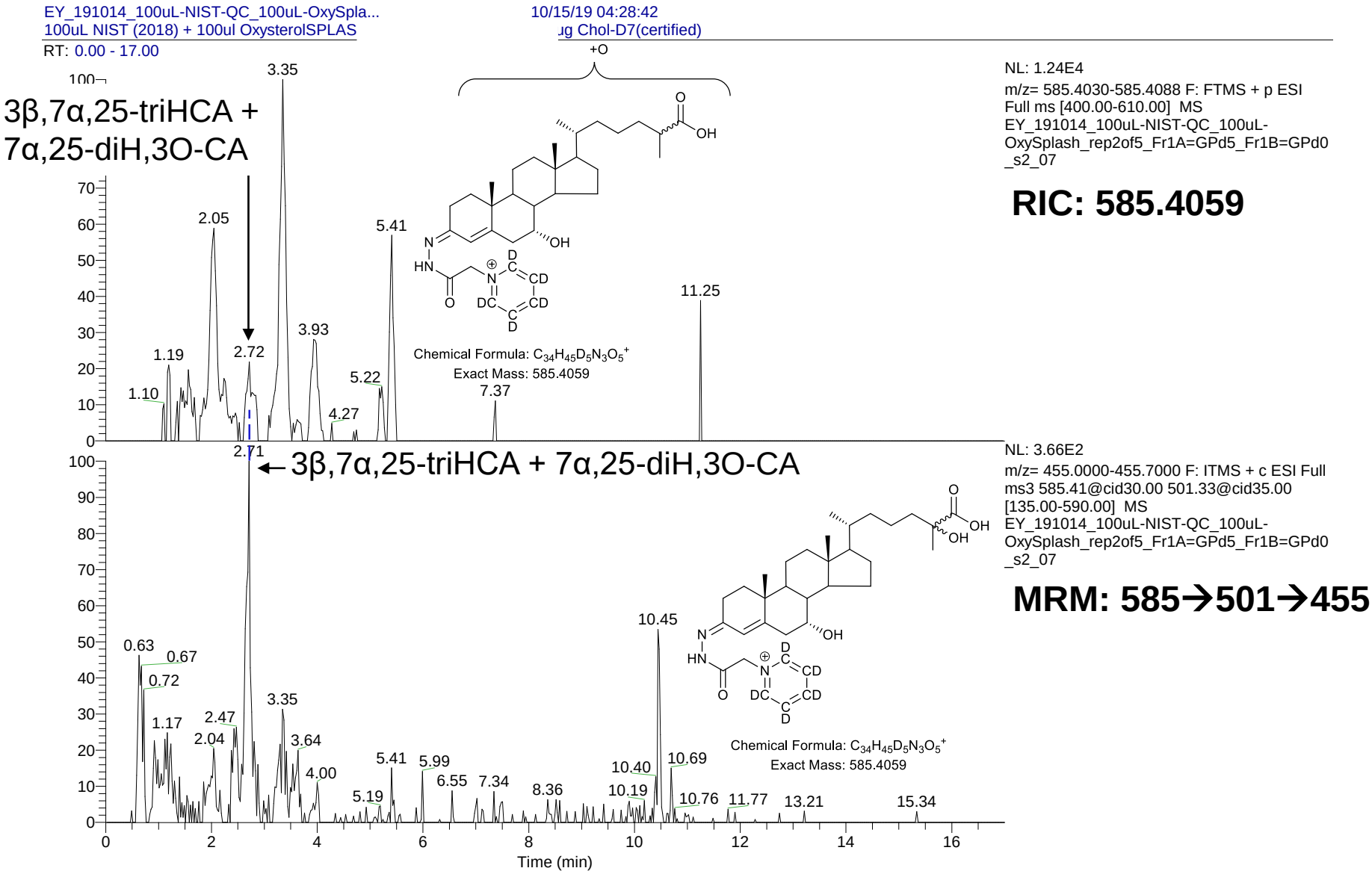
←3β,7α,24-triHCA + 7α,24-diH,3O-CA



NL: 1.58E2
m/z= 427.0000-427.7000 F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07

MRM: 585→501→427

S10G

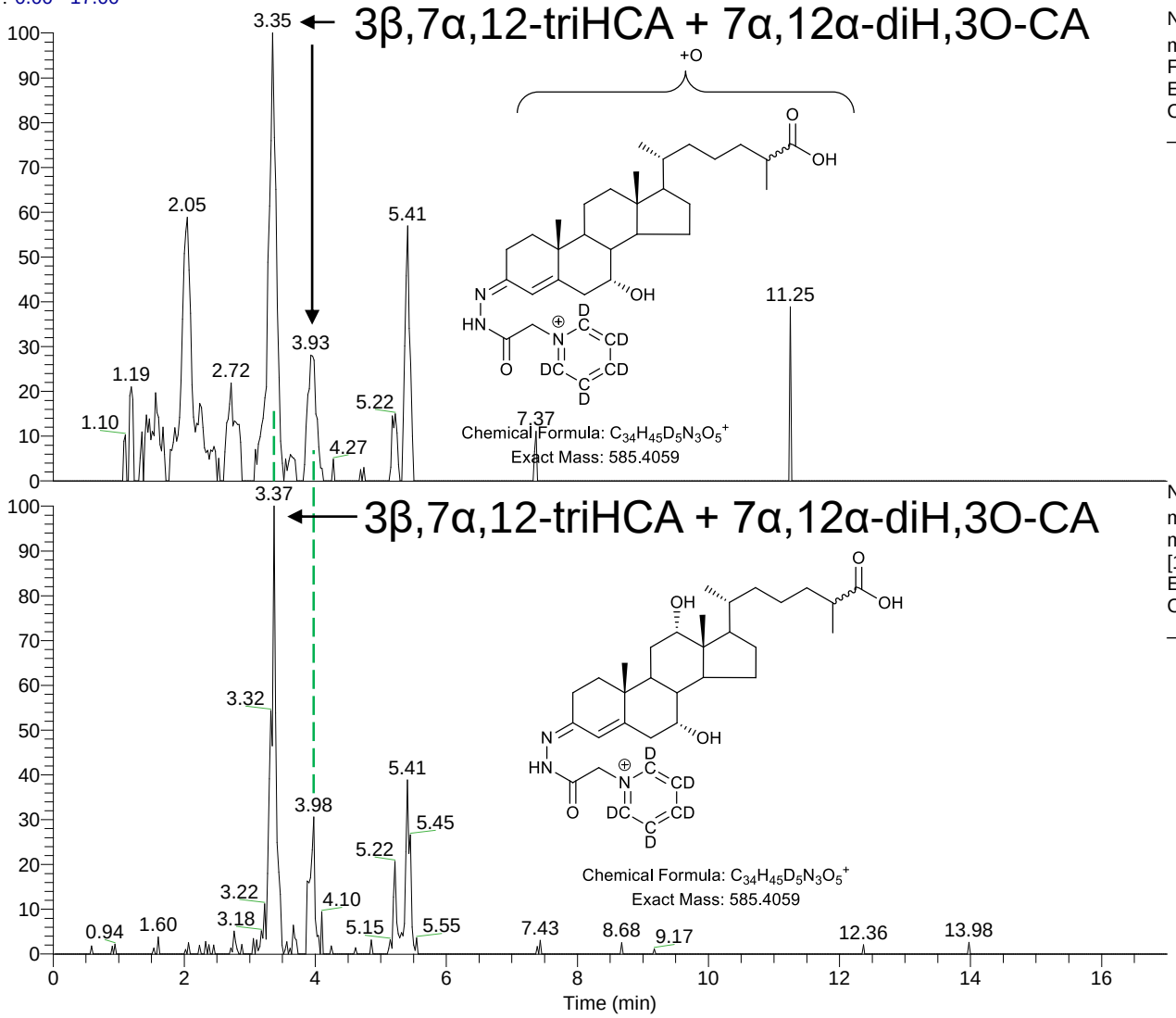

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS
RT: 0.00 - 17.00
10/15/19 04:28:42
ıg Chol-D7(certified)
+O
3β,7α,25-triHCA +
7α,25-diH,3O-CA
3.35
2.05
5.41
11.25
3.93
1.19
2.72
5.22
1.10
7.37
4.27
Chemical Formula: C34H45D5N3O5+
Exact Mass: 585.4059
NL: 1.24E4
m/z= 585.4030-585.4088 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07
RIC: 585.4059
2.71
← 3β,7α,25-triHCA + 7α,25-diH,3O-CA
10.45
0.63
0.67
0.72
3.35
1.17
2.47
2.04
3.64
4.00
5.41
5.99
6.55
7.34
8.36
5.19
10.40
10.19
10.69
10.76
11.77
13.21
15.34
Chemical Formula: C34H45D5N3O5+
Exact Mass: 585.4059
NL: 3.66E2
m/z= 455.0000-455.7000 F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07
MRM: 585→501→455
Time (min)

S10H

EY_191014_100uL-NIST-QC_100uL-OxySpla...
100uL NIST (2018) + 100ul OxysterolSPLAS

10/15/19 04:28:42
ɹg Chol-D7(certified)

RT: 0.00 - 17.00



NL: 1.24E4
m/z= 585.4030-585.4088 F: FTMS + p ESI Full ms [400.00-610.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07

RIC: 585.4059

NL: 3.75E2
m/z= 422.0000-422.7000 F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.00] MS EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1B=GPd0_s2_07

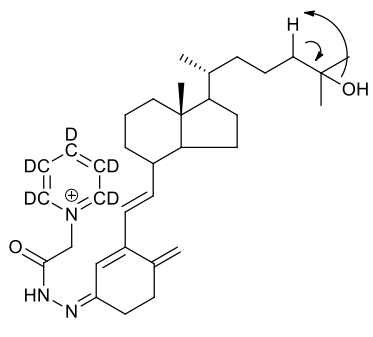
MRM: 585→501→422

11A

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 05:10:14
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 537.42@cid30.00 435.34@cid35.00 [115.00-545.0

8.19 AV: 1 NL: 4.20E3

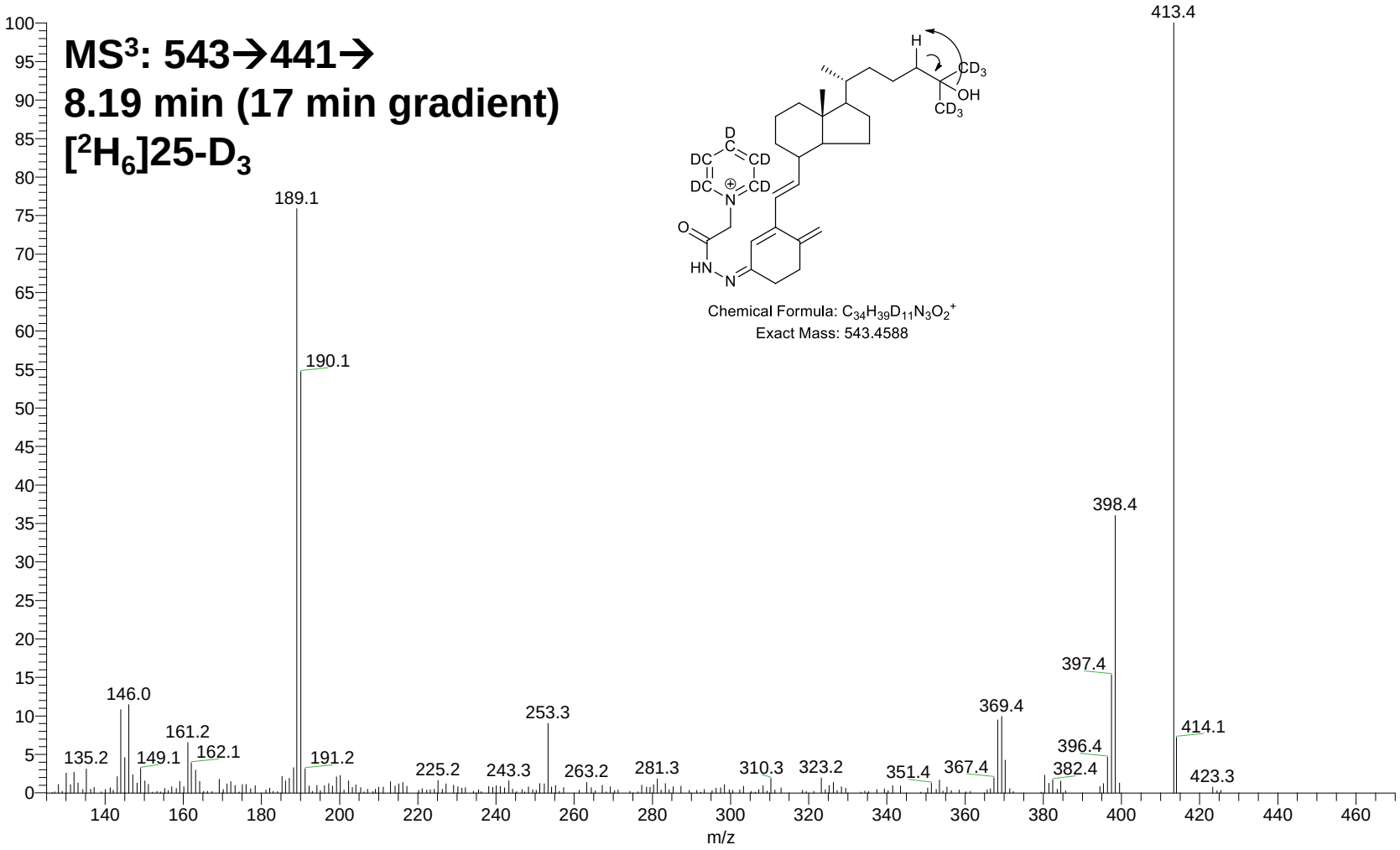
MS^3: 537→435→
8.19 min (17 min gradient)
25-D$_3$



Chemical Formula: $C_{34}H_{45}D_5N_3O_2^+$
Exact Mass: 537.4211

11B

EY_191014_100uL-NIST-QC_with_2017-Pro... 10/16/19 13:46:25
100uL NIST (2018) + 2017-Protocol-iSTDs (20ng 24RS-HC-d6-cert) + 10ng 7(certified)
EY_191014_100uL-NIST-QC_with_2017-Protocol-iSTDs_rep2of5_Fr1A=GF 50 RT: 8.19 AV: 1 NL: 1.28E4
F: ITMS + c ESI Full ms3 543.46@cid30.00 441.37@cid35.00 [120.00-550.0

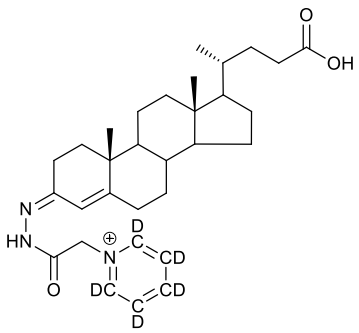


11C

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 05:51:45
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 511.37@cid30.00 427.30@cid35.00 [115.00-515.0

.35 AV: 1 NL: 6.55E2

MS^3: 511→427→
3.35 min (17 min gradient)
3βH-Δ^5-BA



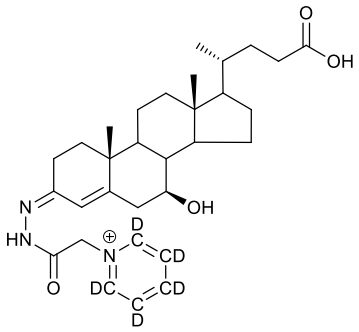
Chemical Formula: $C_{31}H_{39}D_5N_3O_3^+$
Exact Mass: 511.3691

11D

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:07:57
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 527.36@cid30.00 443.29@cid35.00 [120.00-535.0

.12 AV: 1 NL: 1.08E3

MS^3: 527→443→
1.12 min (17 min gradient)
3β,7β-diH-Δ^5-BA

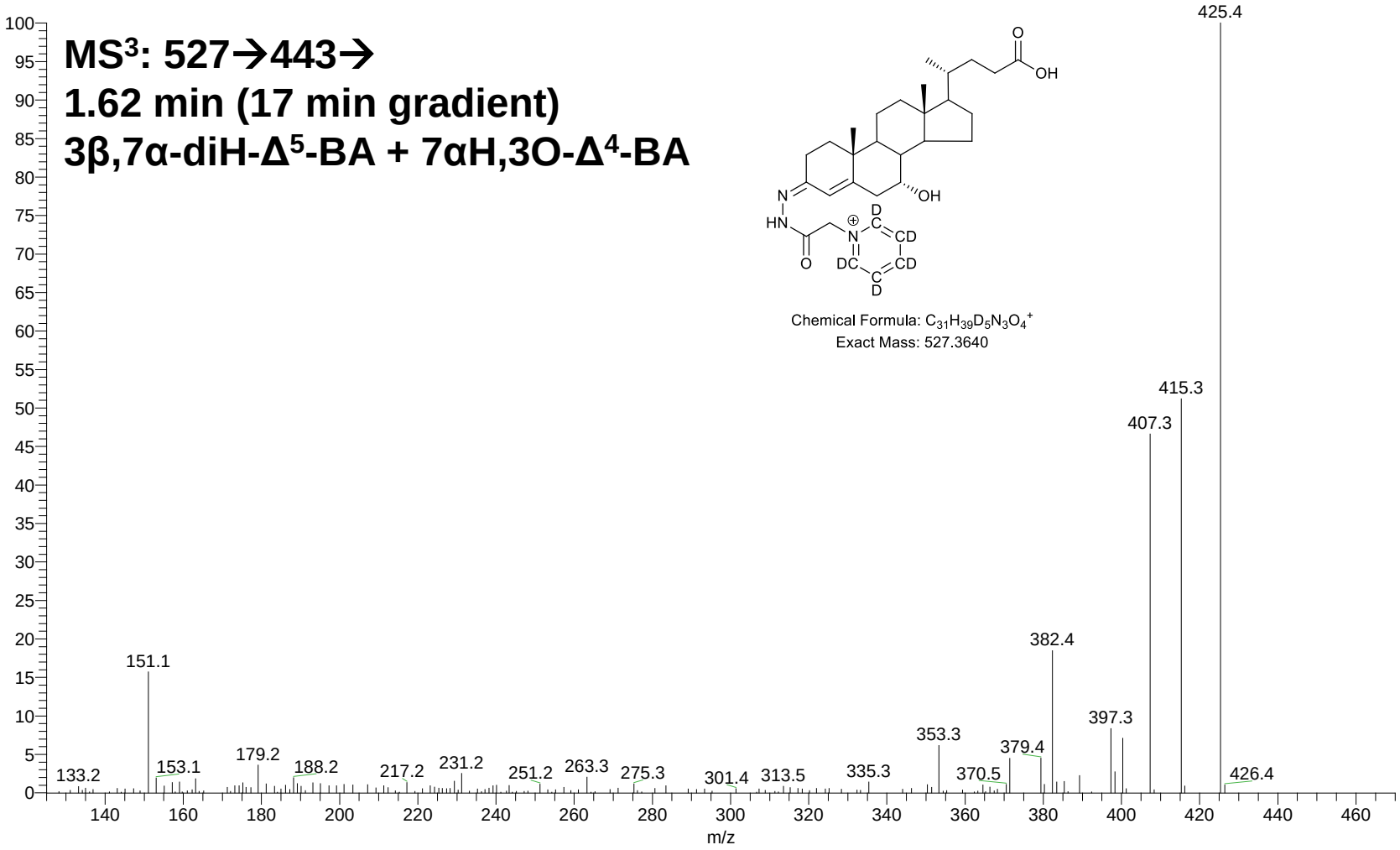


Chemical Formula: $C_{31}H_{39}D_5N_3O_4^+$
Exact Mass: 527.3640

11E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:07:57
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 527.36@cid30.00 443.29@cid35.00 [120.00-535.0

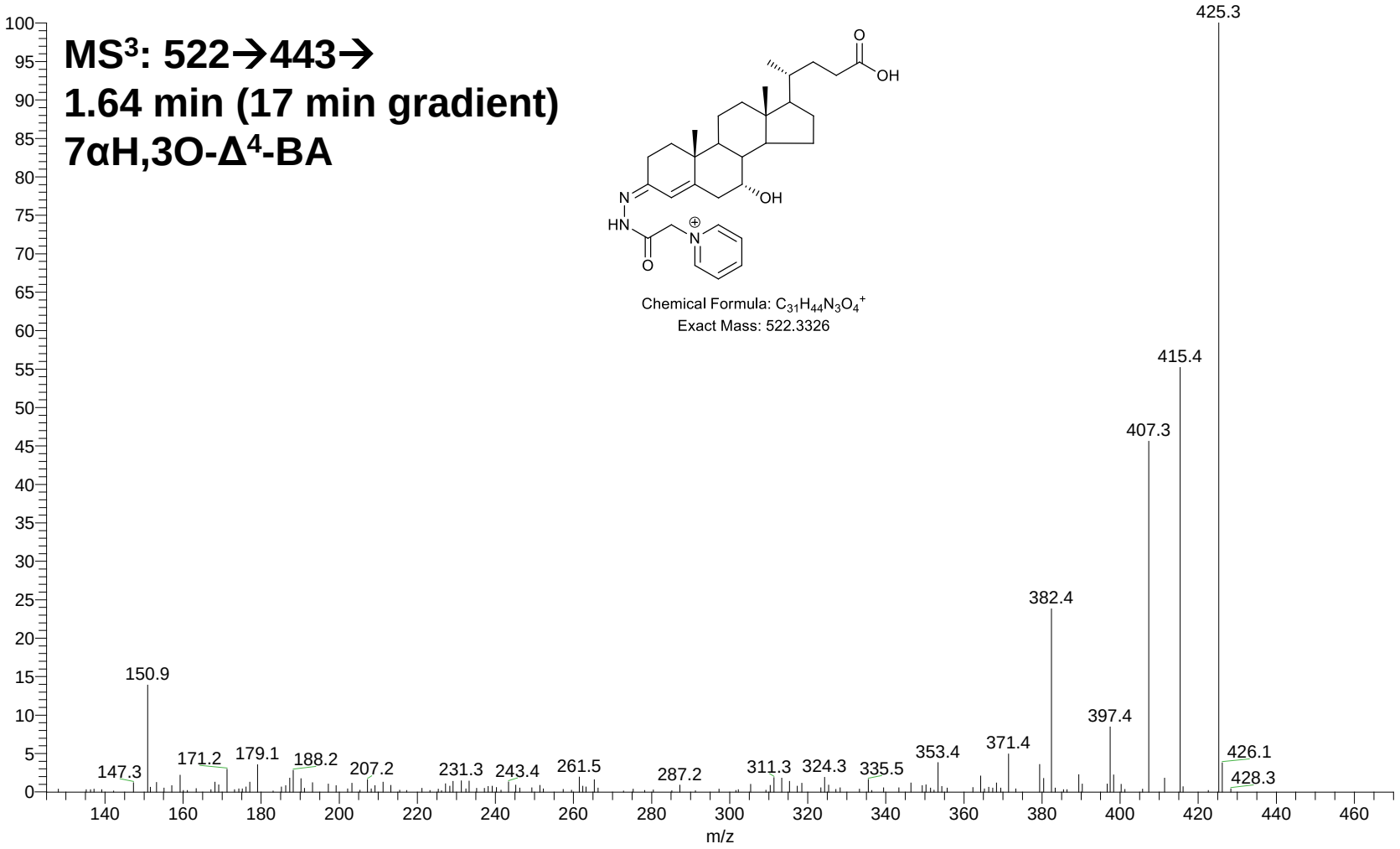
.62 AV: 1 NL: 3.59E3



11F

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:07:57
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 522.33@cid30.00 443.29@cid35.00 [120.00-535.0

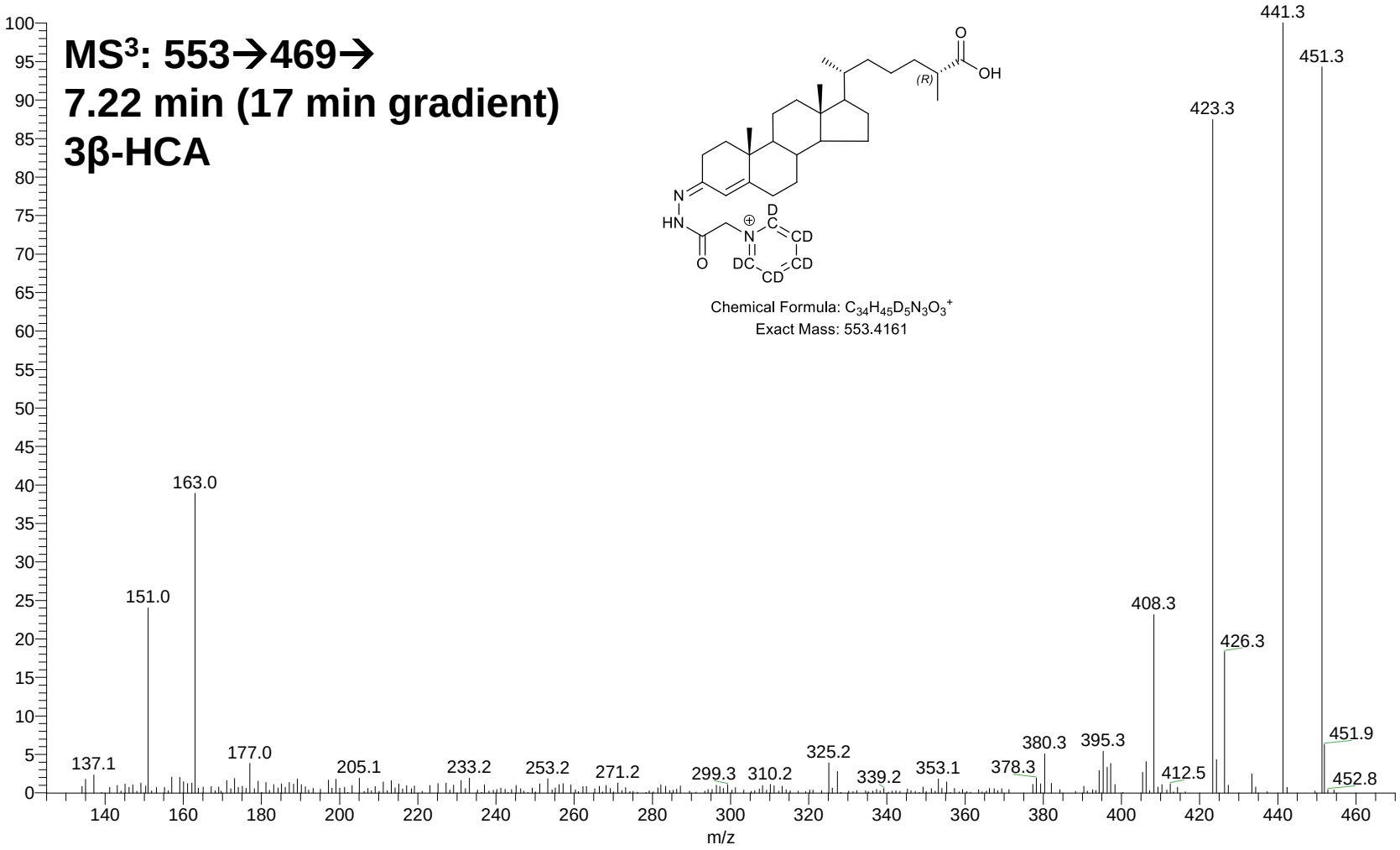
.64 AV: 1 NL: 2.12E3



11G

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 553.42@cid30.00 469.34@cid35.00 [125.00-560.0

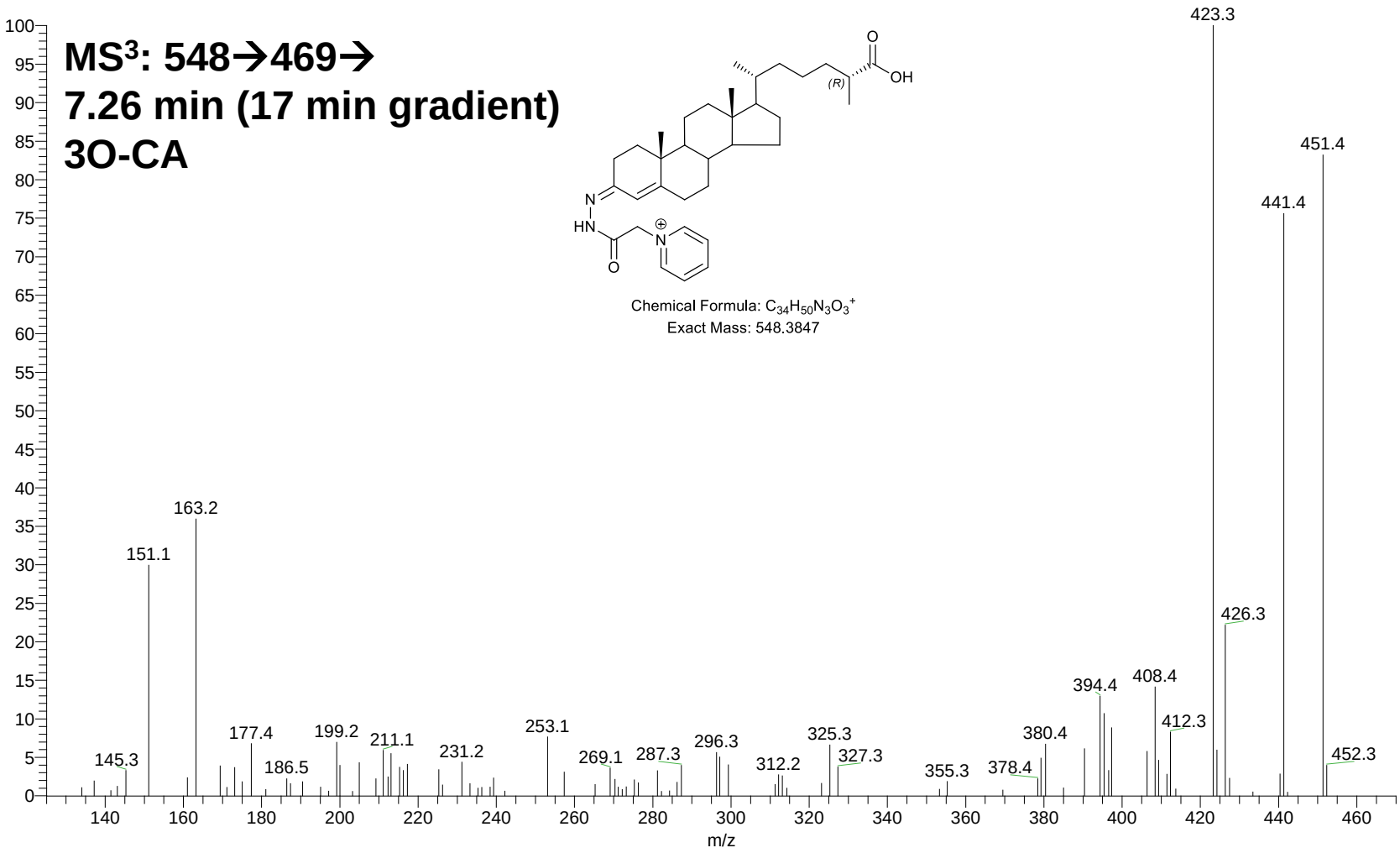
7.22 AV: 1 NL: 3.11E4



11H

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 548.38@cid30.00 469.34@cid35.00 [125.00-560.0

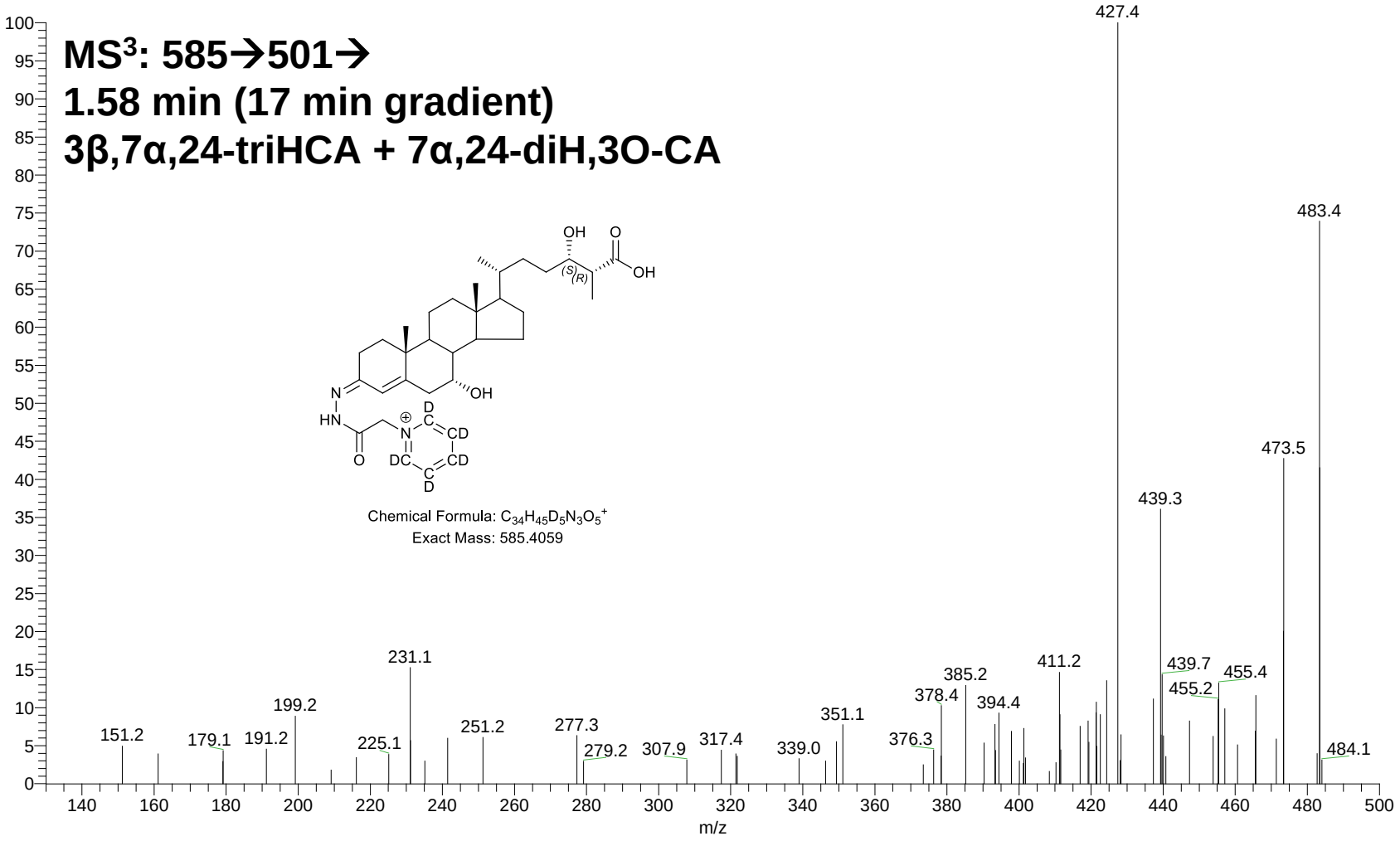
7.26 AV: 1 NL: 4.64E2



S12A

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.0

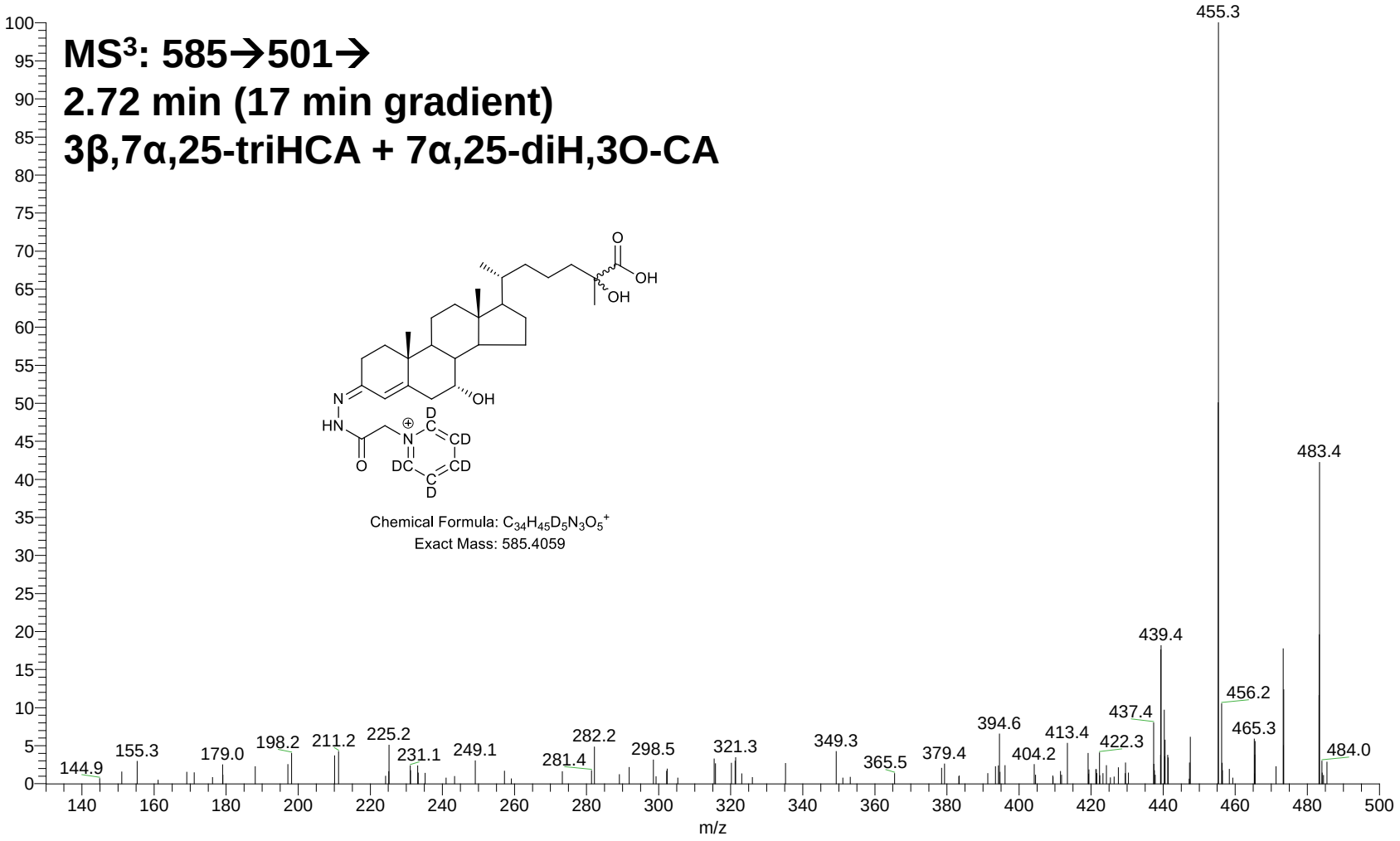
T: 1.56-1.60 AV: 3 NL: 5.27E1



S12B

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.0

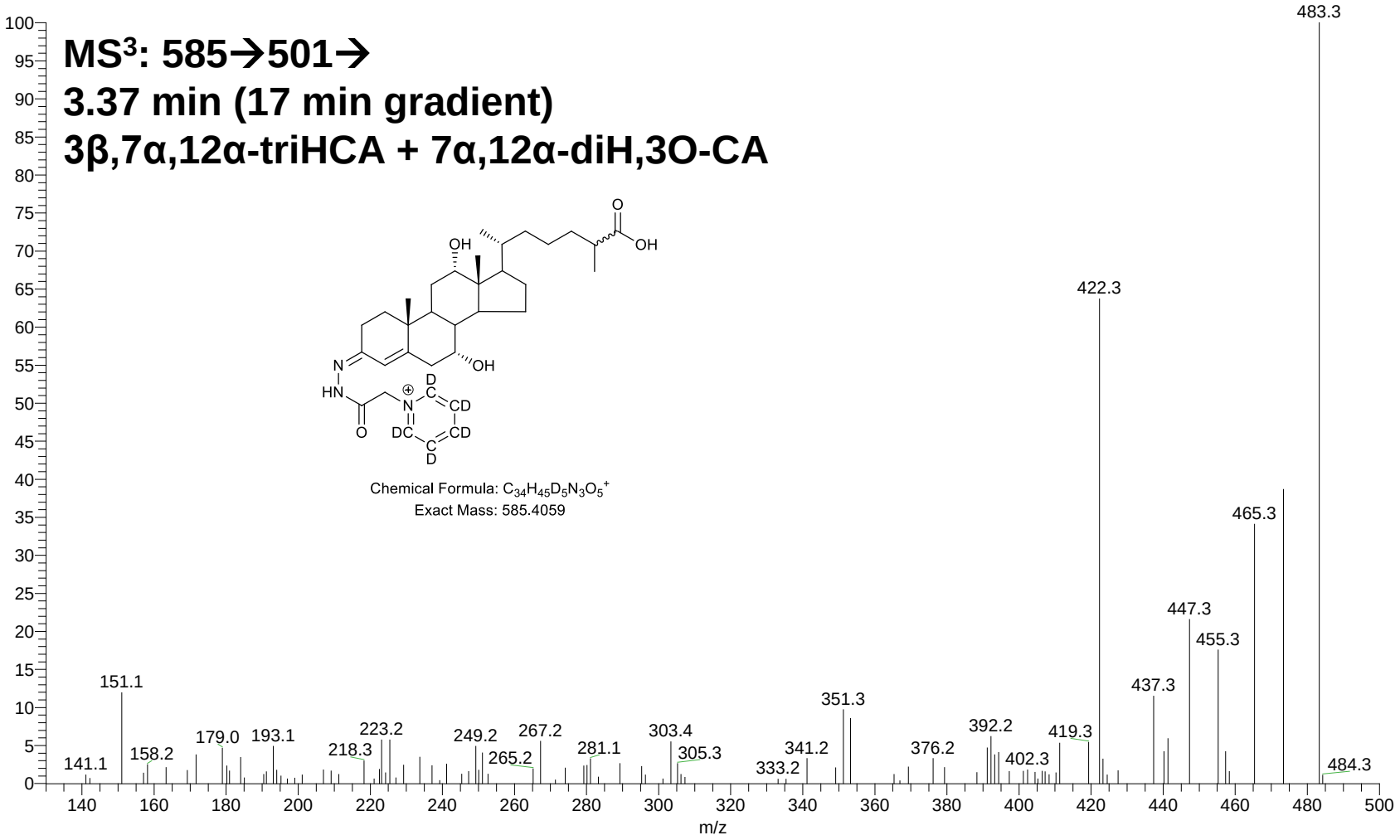
T: 2.64-2.76 AV: 6 NL: 7.96E1



12C

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.0

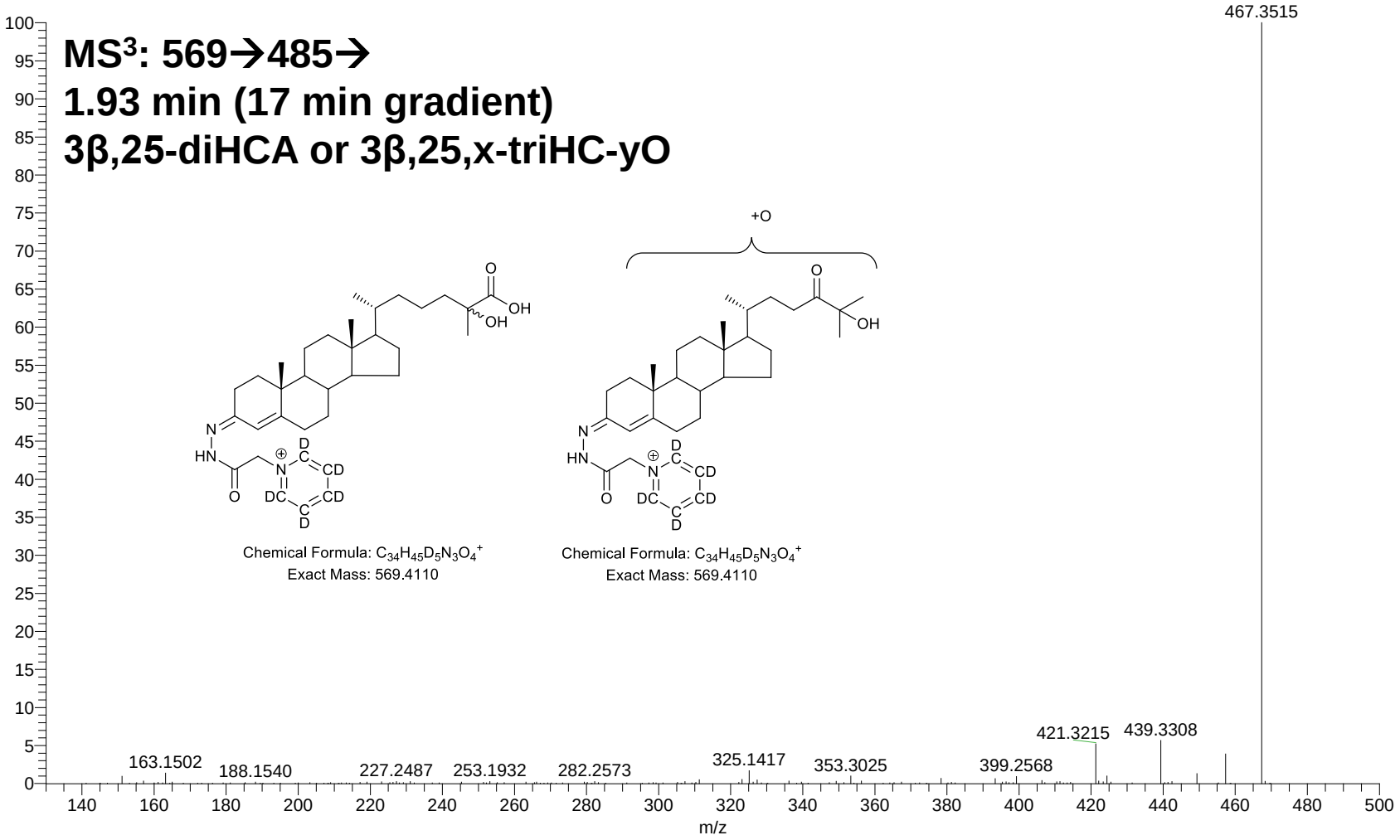
.37 AV: 1 NL: 5.89E2



S13A

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

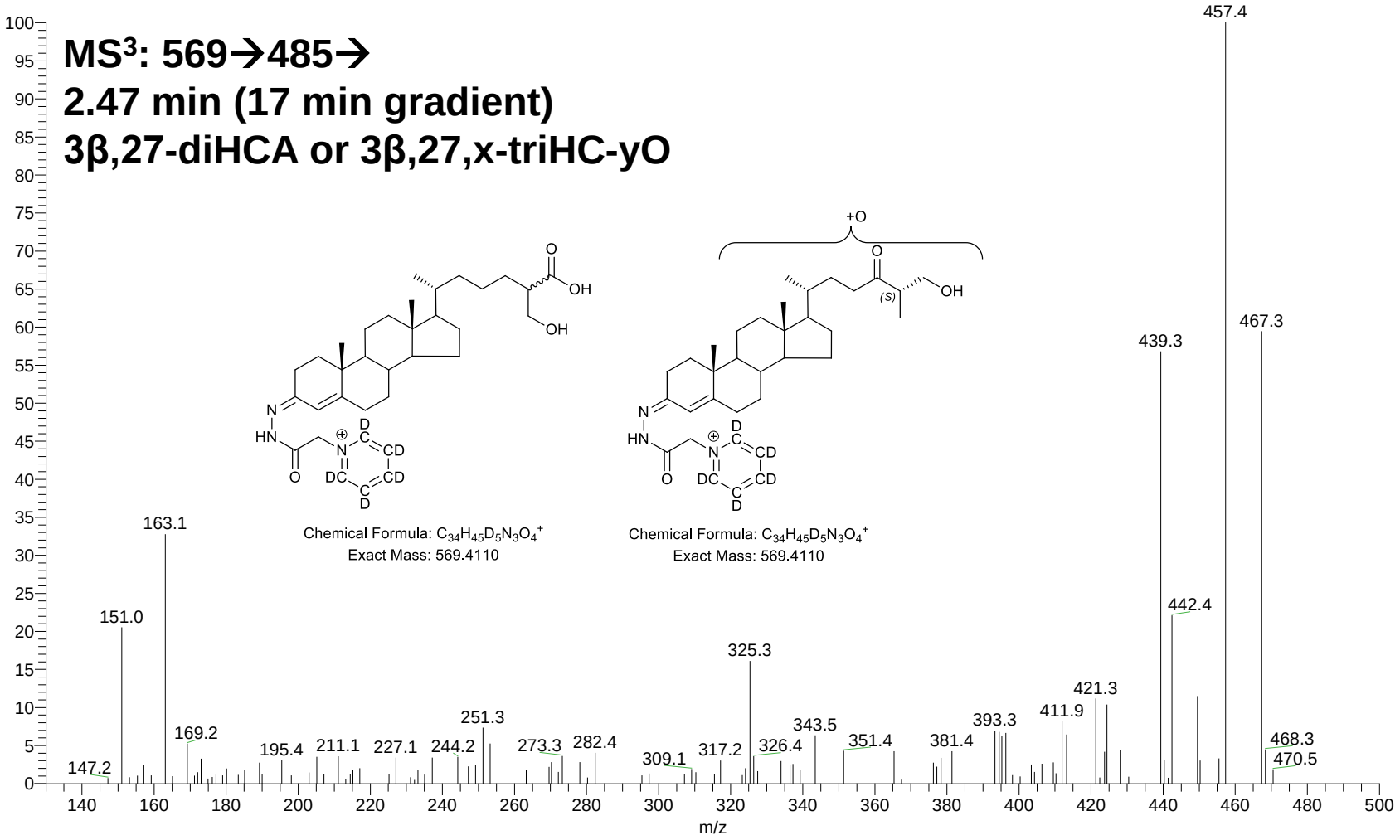
.93 AV: 1 NL: 1.39E4



S13B

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

.47 AV: 1 NL: 5.48E2

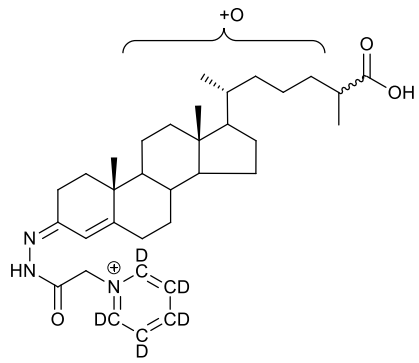


S13C

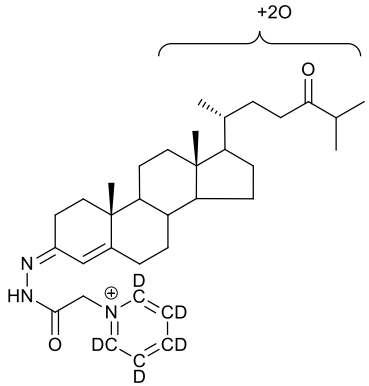
EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

.97 AV: 1 NL: 1.70E2

MS³: 569→485→
3.97 min (17 min gradient)
3β,x-diHCA or 3β,x,y-triHC-zO



Chemical Formula: $C_{34}H_{45}D_5N_3O_4^+$
Exact Mass: 569.4110



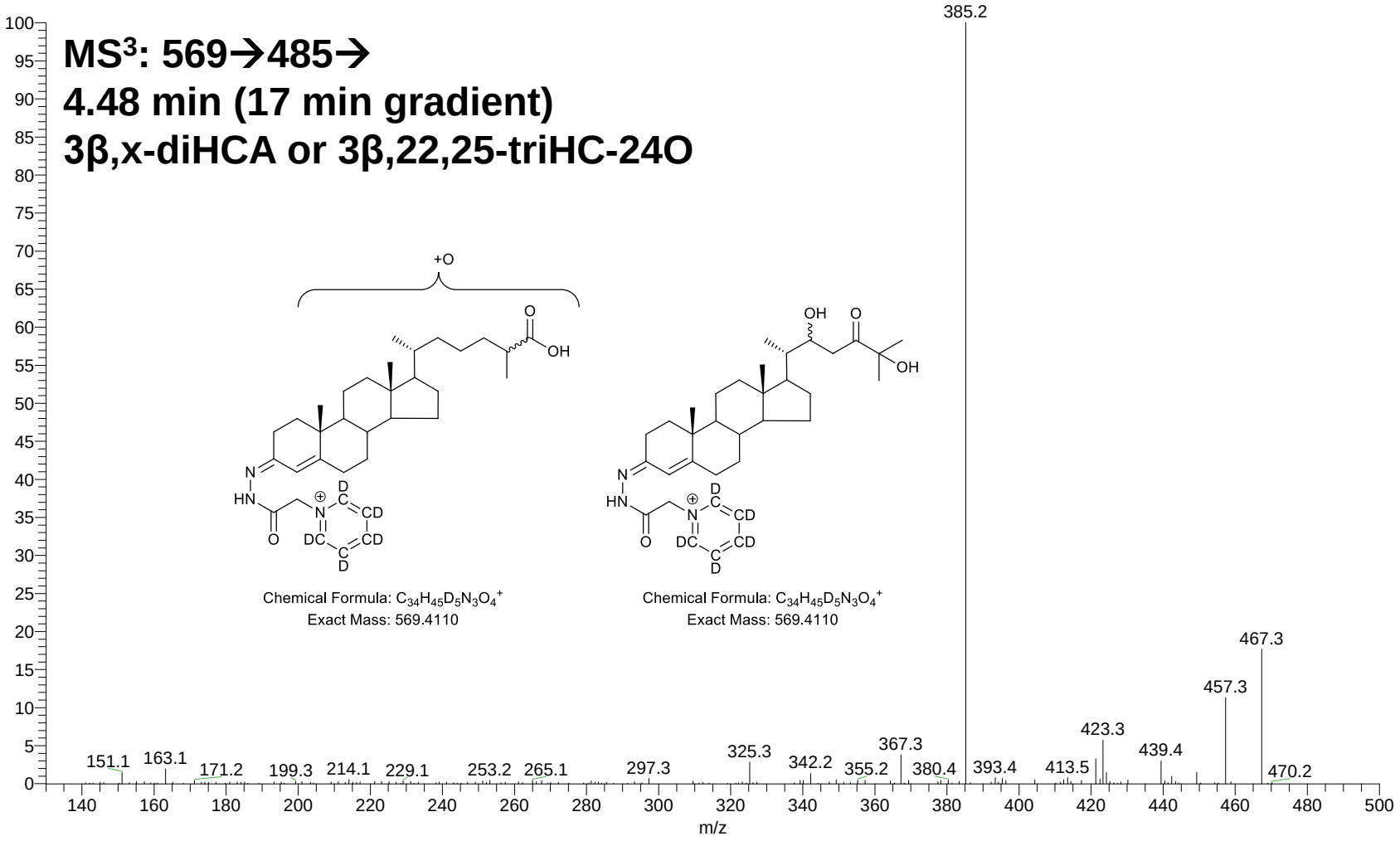
Chemical Formula: $C_{34}H_{45}D_5N_3O_4^+$
Exact Mass: 569.4110

S13D

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 03:47:11
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 569.41@cid30.00 485.34@cid35.00 [130.00-575.0

.48 AV: 1 NL: 6.92E3

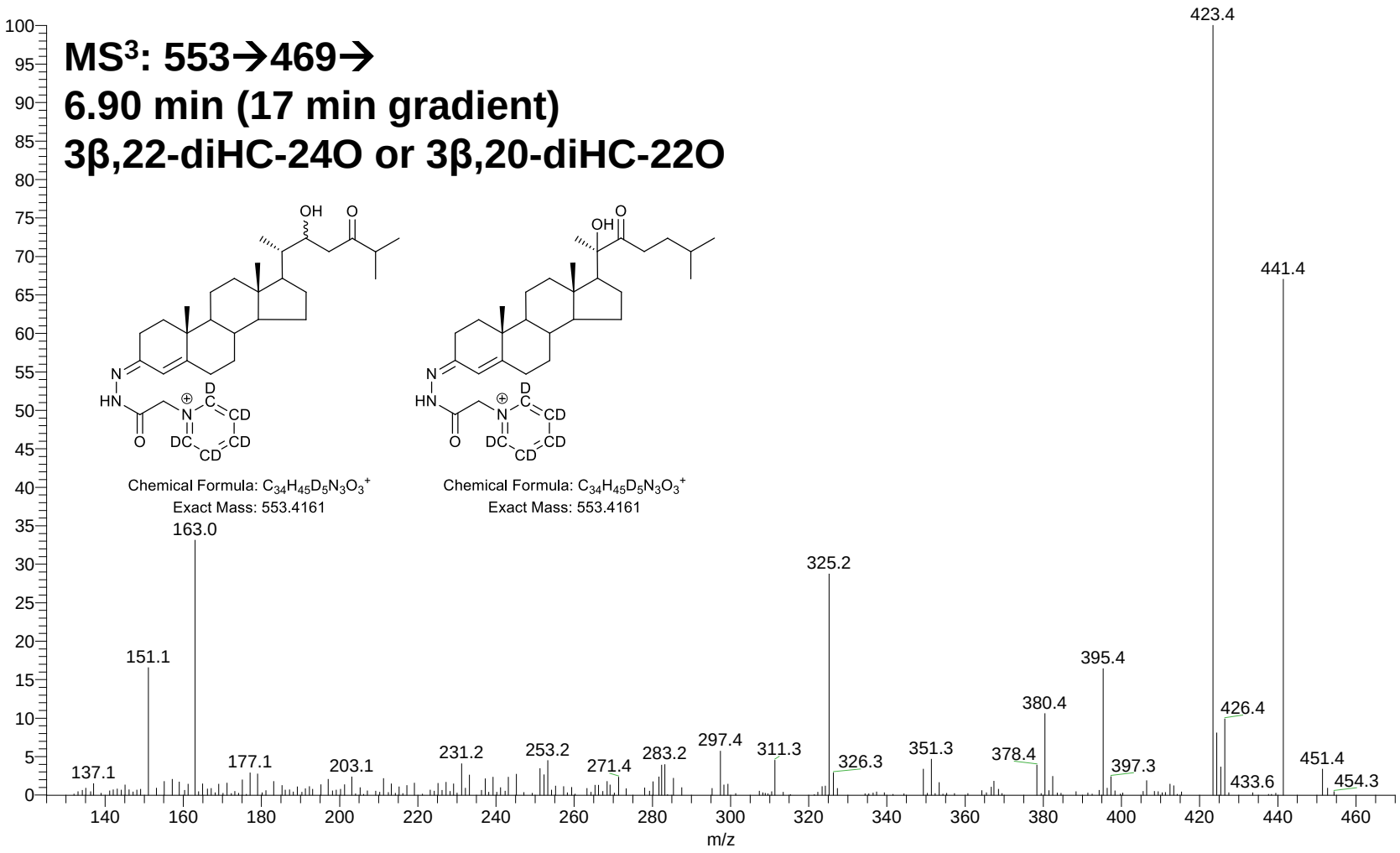
MS^3: 569→485→
4.48 min (17 min gradient)
3β,x-diHCA or 3β,22,25-triHC-24O



S13E

EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 553.42@cid30.00 469.34@cid35.00 [125.00-560.0

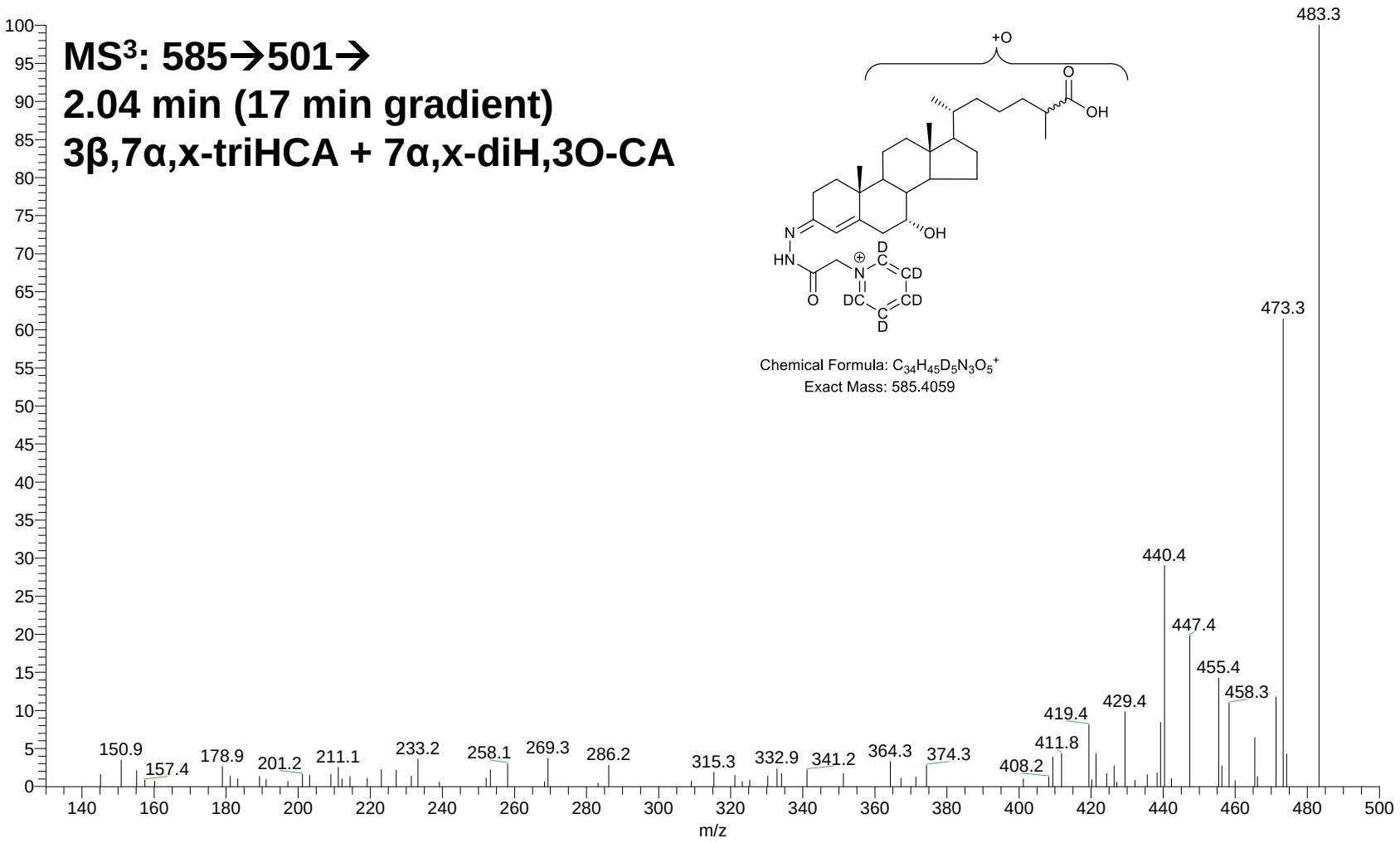
6.90 AV: 1 NL: 4.69E3



S13F

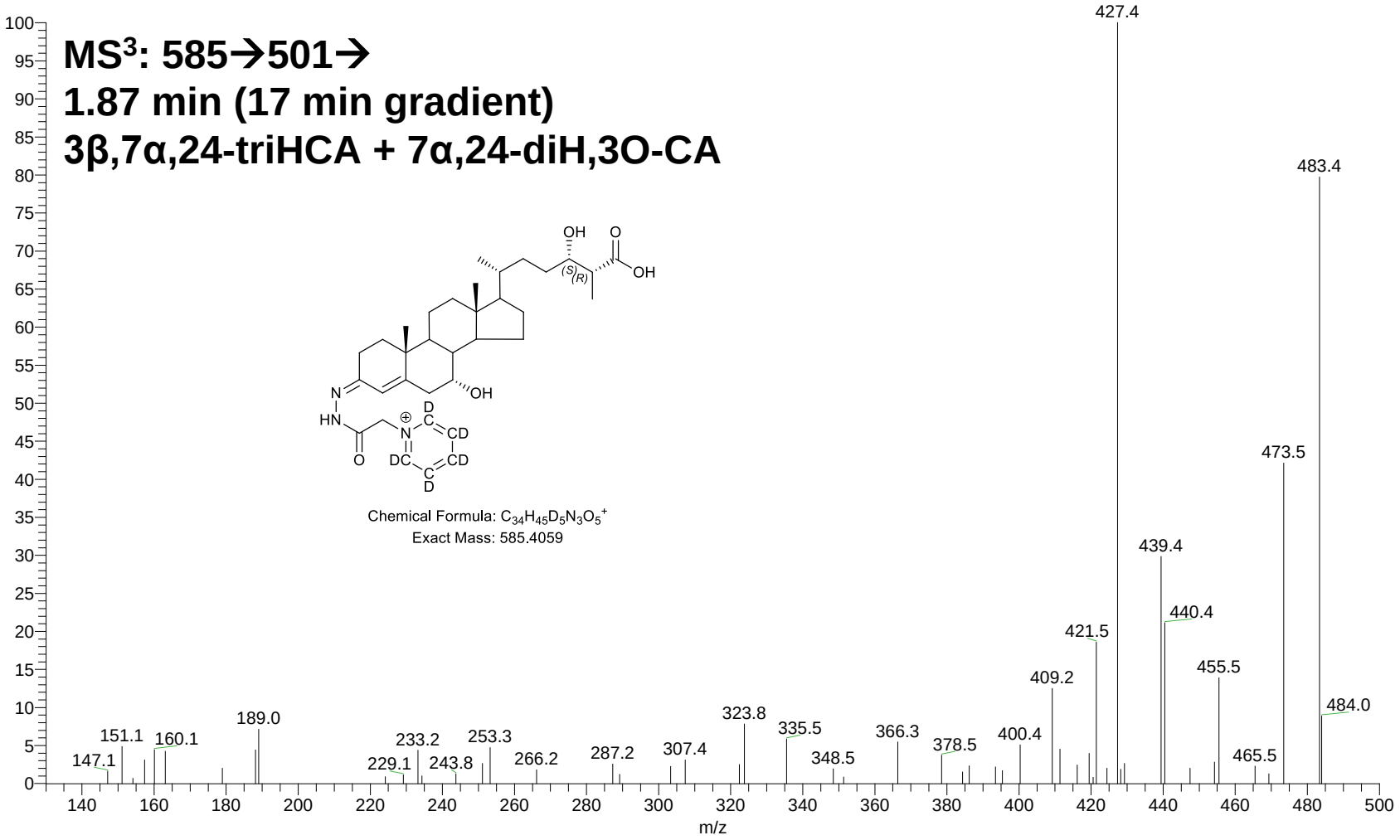
EY_191014_100uL-NIST-QC_100uL-OxySpla... 10/15/19 04:28:42
100uL NIST (2018) + 100ul OxysterolSPLASH + 10ng 22S-HCO-D7 + 20ug
EY_191014_100uL-NIST-QC_100uL-OxySplash_rep2of5_Fr1A=GPd5_Fr1E
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.0

.04 AV: 1 NL: 5.27E2



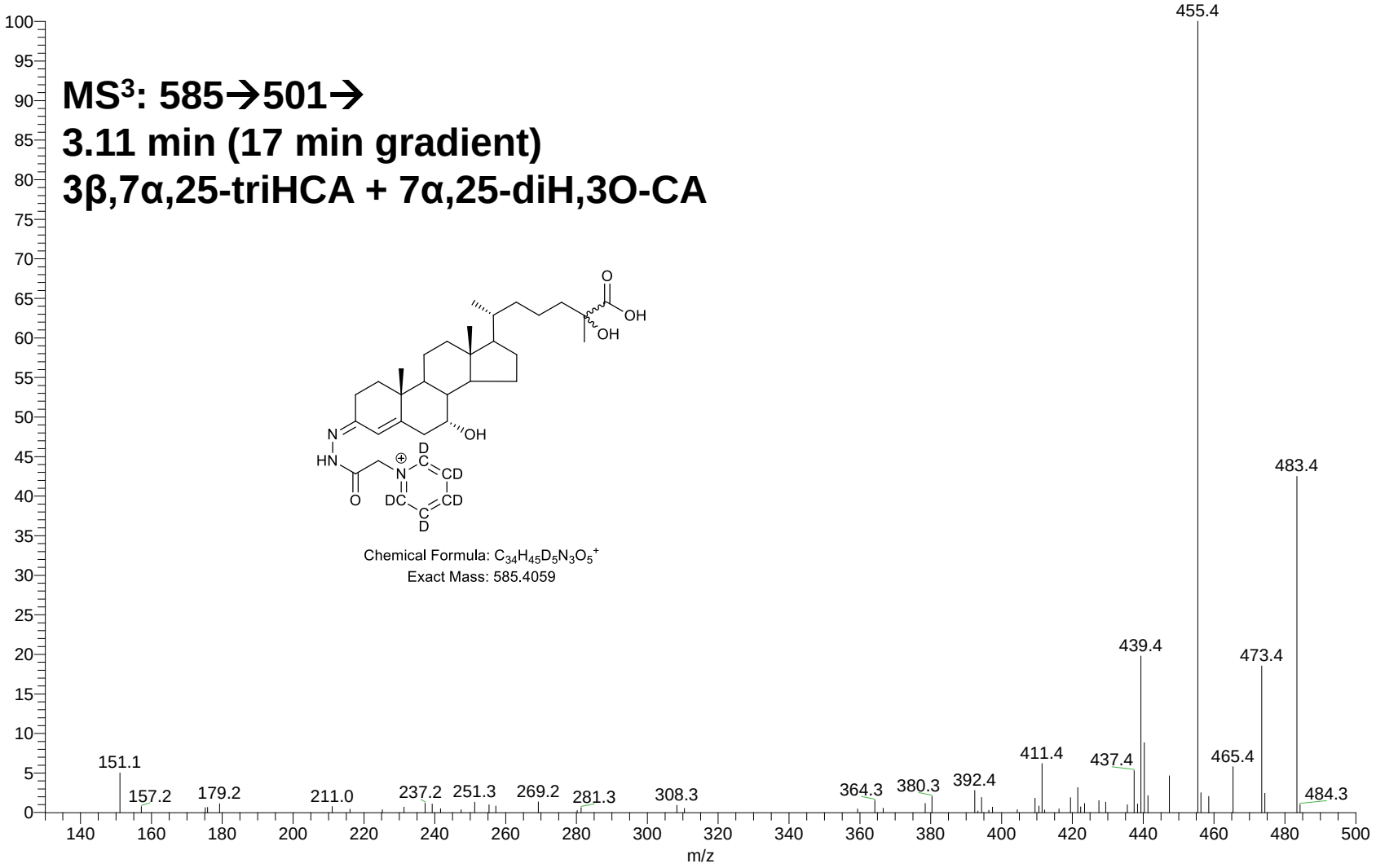
S14A

EY_191029_100uL-Cambridge-CSF-pool_20... 10/29/19 21:38:19
100uL Cambridge Pooled CSF (2019) + 20ul OxysterolSPLASH + 1ng 22S-H tified)
EY_191029_100uL-Cambridge-CSF-pool_20uL-OxySPLASH_r2_NON-HYD A=GPd5_Fr1B=GPd0_s2_neat_07 #425 RT: 1.87 AV: 1 NL: 3.01E2
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-590.0



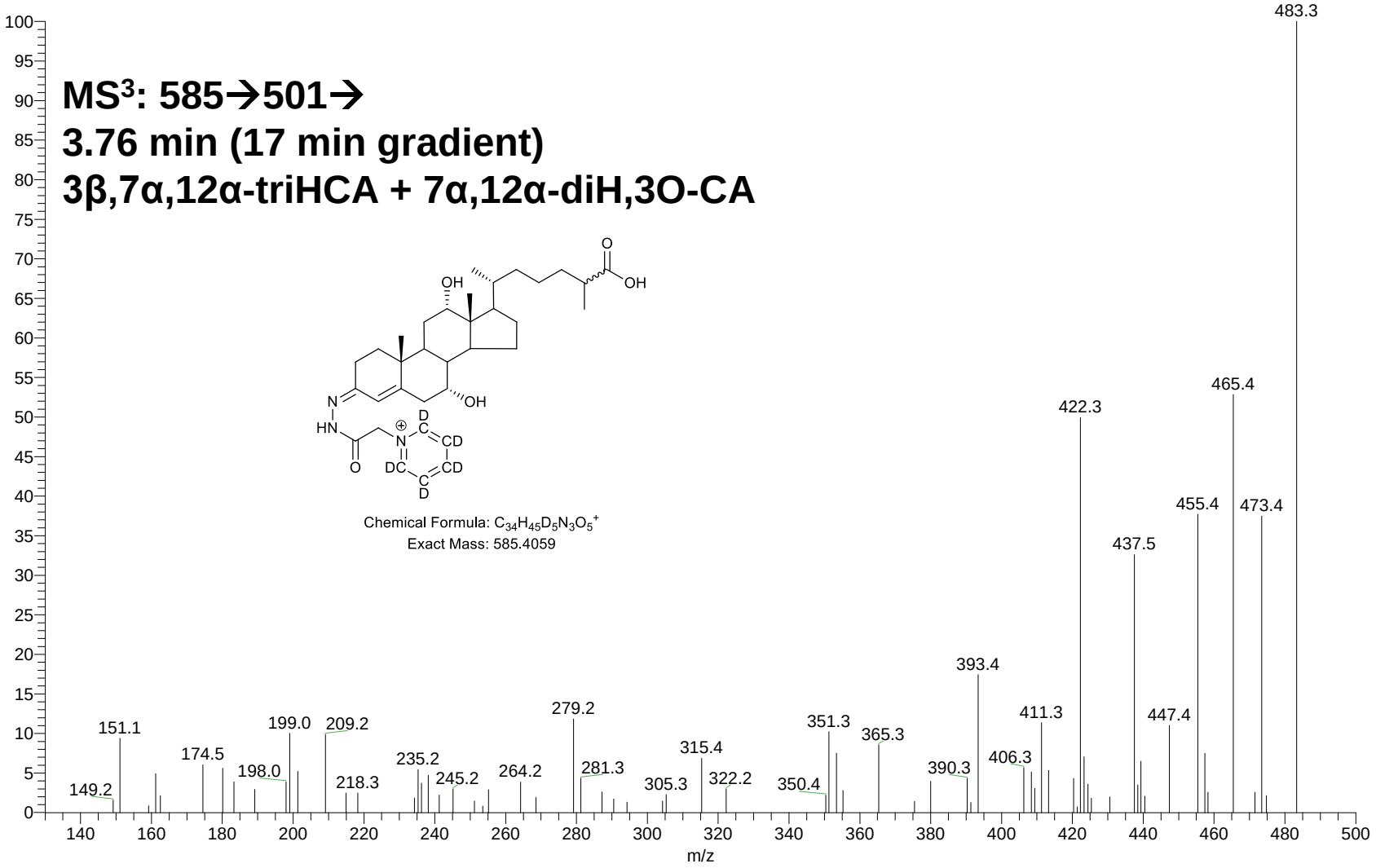
S14B

EY_191029_100uL-Cambridge-CSF-pool_20... 10/29/19 21:38:19
100uL Cambridge Pooled CSF (2019) + 20ul OxysterolSPLASH + 1ng 22 certified)
EY_191029_100uL-Cambridge-CSF-pool_20uL-OxySPLASH_r2_NON-H r1A=GPd5_Fr1B=GPd0_s2_neat_07 #700 RT: 3.11 AV: 1 NL: 1.34E3
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-59



S14C

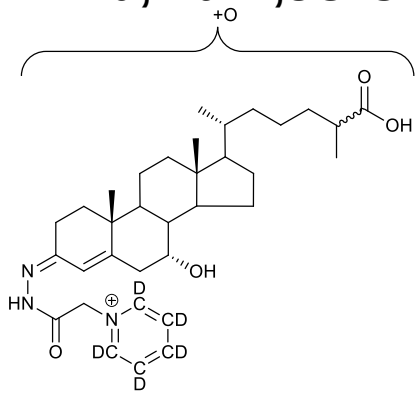
EY_191029_100uL-Cambridge-CSF-pool_20... 10/29/19 21:38:19
100uL Cambridge Pooled CSF (2019) + 20ul OxysterolSPLASH + 1ng 22 certified)
EY_191029_100uL-Cambridge-CSF-pool_20uL-OxySPLASH_r2_NON-H r1A=GPd5_Fr1B=GPd0_s2_neat_07 #845 RT: 3.76 AV: 1 NL: 2.89E2
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-59



S14D

EY_191029_100uL-Cambridge-CSF-pool_20... 10/29/19 21:38:19
100uL Cambridge Pooled CSF (2019) + 20ul OxysterolSPLASH + 1ng 22 certified)
EY_191029_100uL-Cambridge-CSF-pool_20uL-OxySPLASH_r2_NON-H r1A=GPd5_Fr1B=GPd0_s2_neat_07 #540 RT: 2.39 AV: 1 NL: 3.45E3
F: ITMS + c ESI Full ms3 585.41@cid30.00 501.33@cid35.00 [135.00-59

MS^3: 585→501→
2.39 min (17 min gradient)
3β,7α,x-triHCA + 7α,x-diH,3O-CA



Chemical Formula: $C_{34}H_{45}D_5N_3O_5^+$
Exact Mass: 585.4059