Structure–metabolism relationships of 4-pentenyl synthetic cannabinoid receptor agonists using *in vitro* human hepatocyte incubations and high-resolution mass spectrometry

Supplementary Information for Archives of Toxicology – Chromatograms and Mass Spectra

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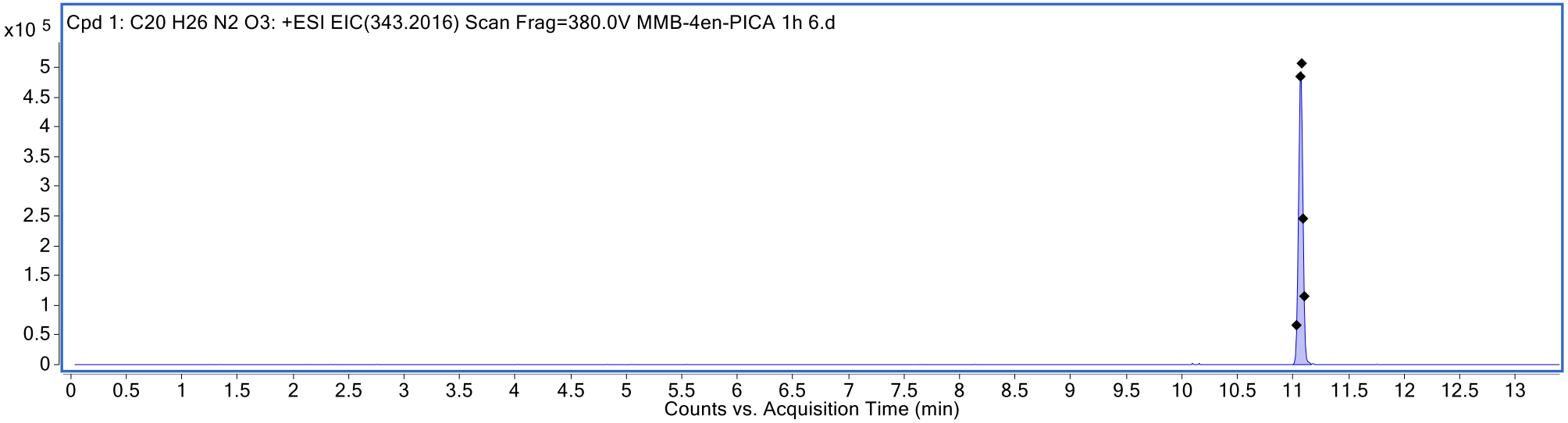
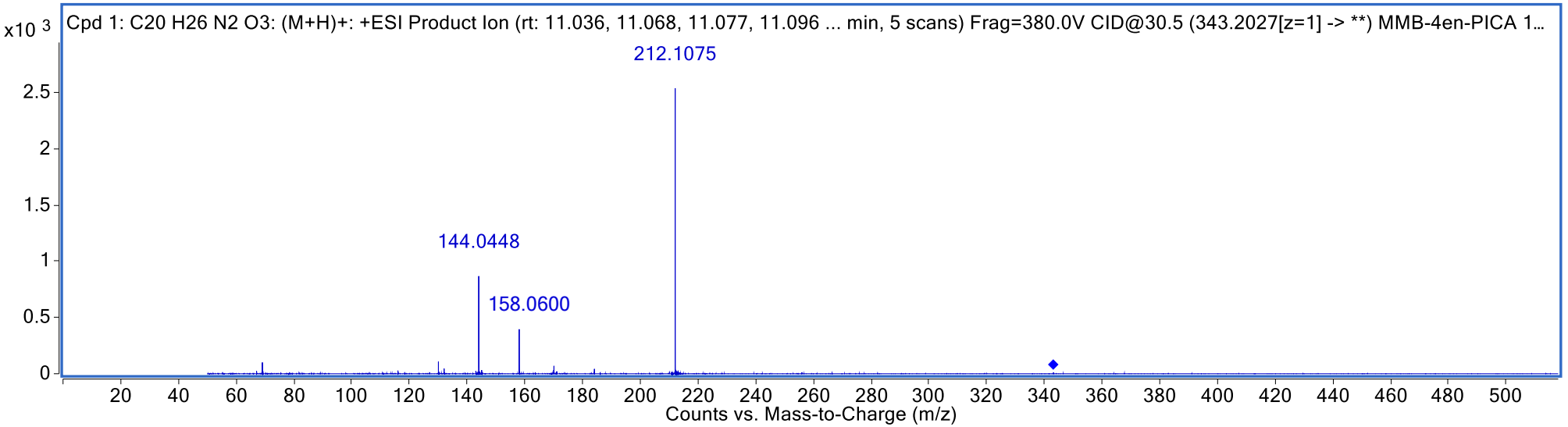
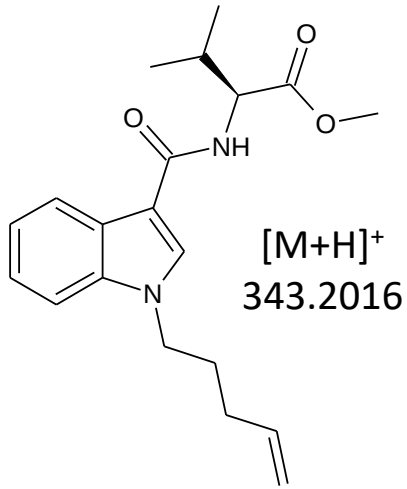
MMB-4en-PICA

Metabolism

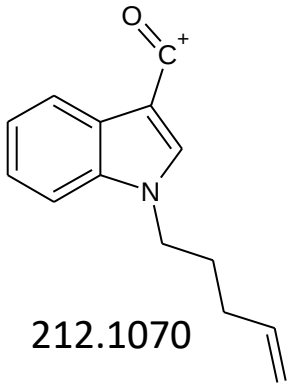
MMB-4en-PICA, RT 11.07 min, *m/z* 343.2038







[M+H]+
343.2016



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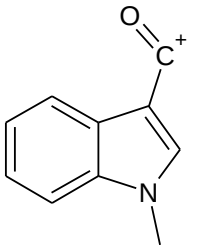

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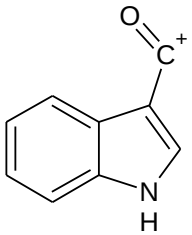
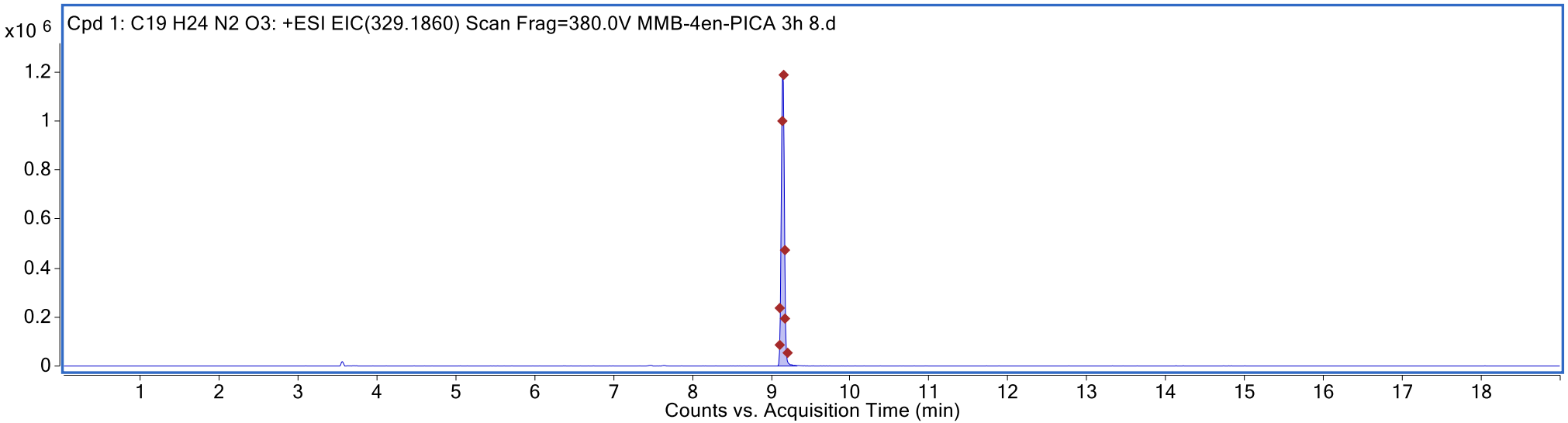
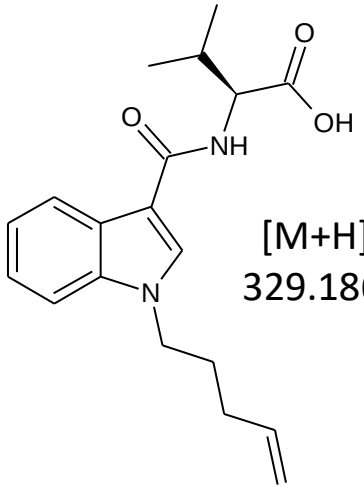


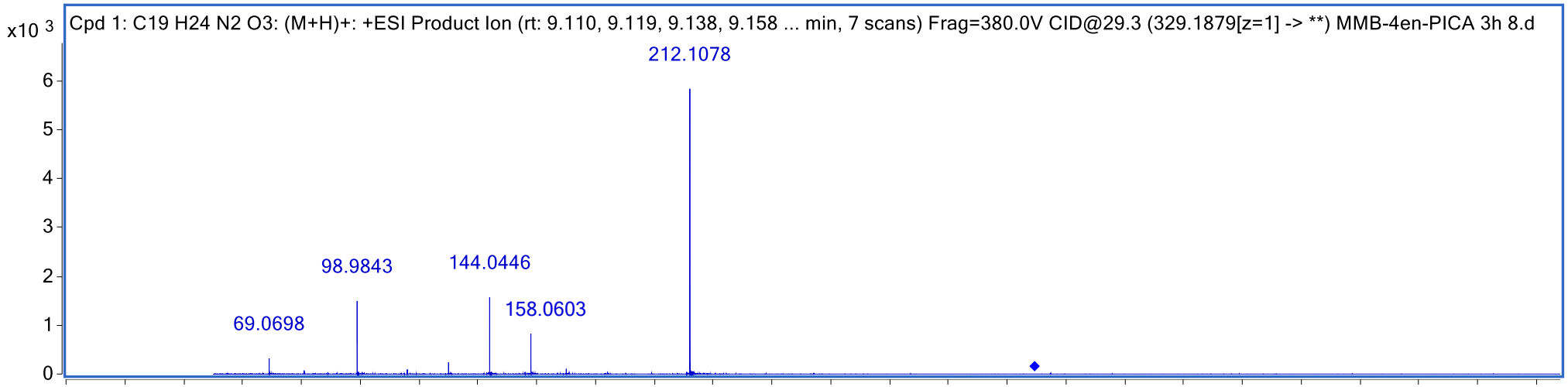
144.0444

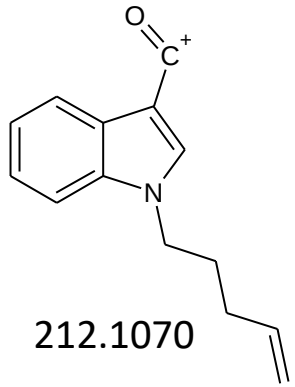
A1, Ester hydrolysis, RT 9.15 min, *m/z* 329.1879





[M+H]+
329.1860





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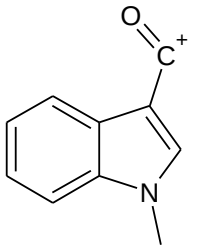

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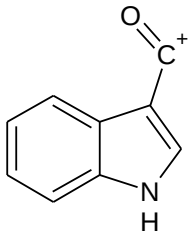

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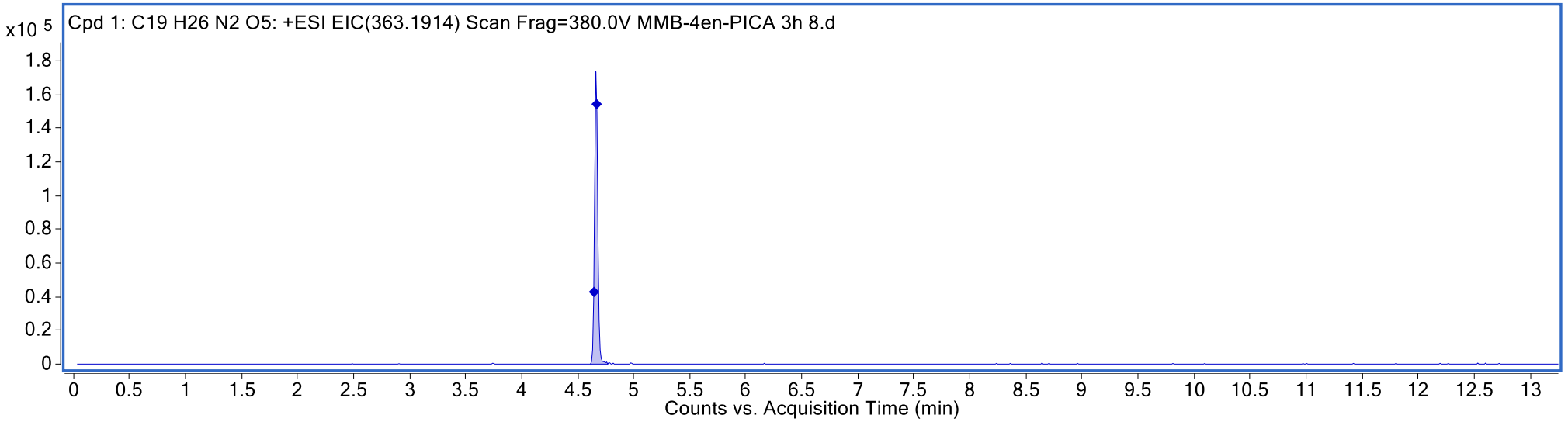
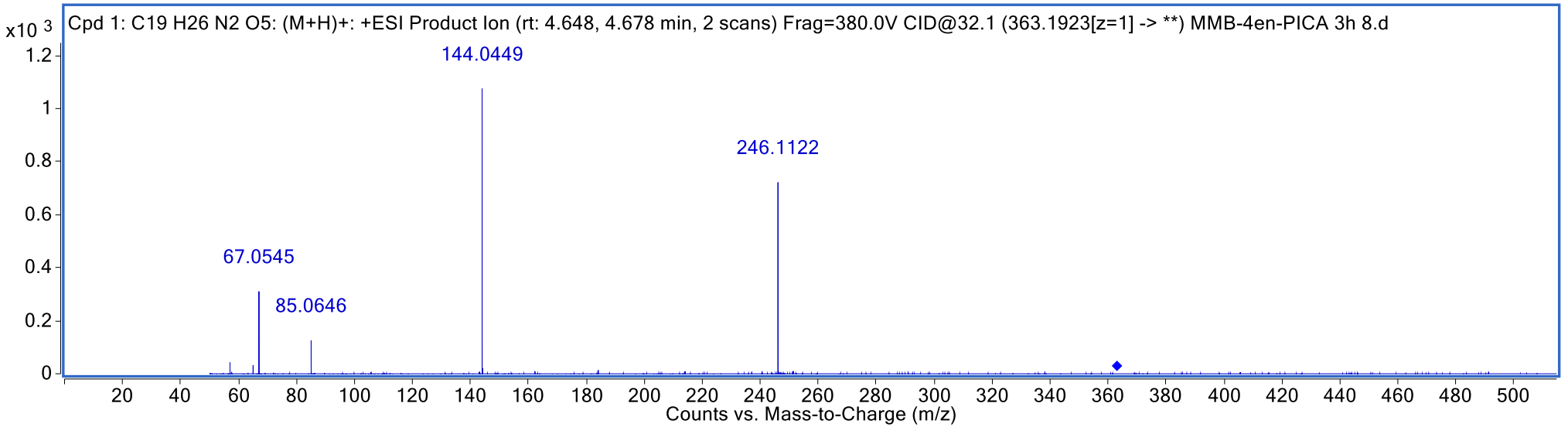
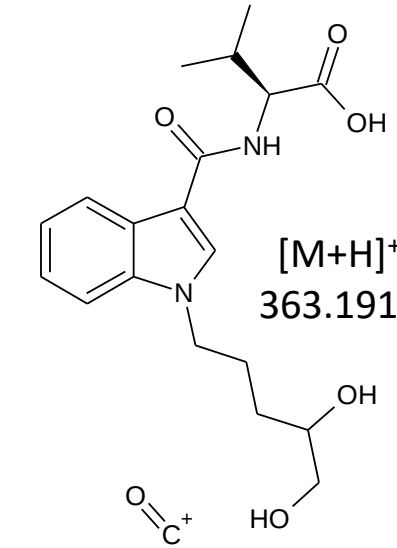
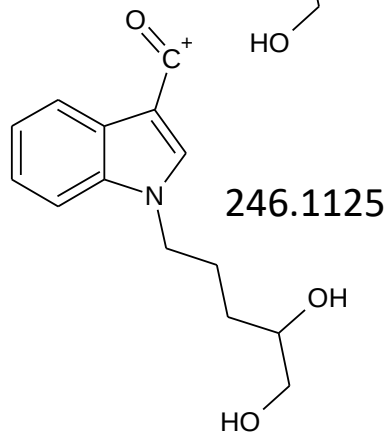
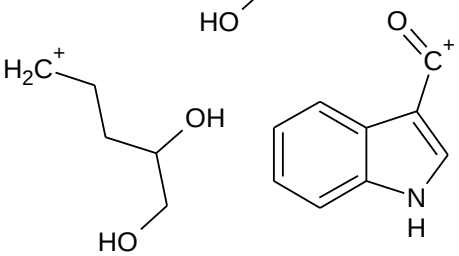
A2, Ester hydrolysis + dihydrodiol formation, RT 4.66 min, *m/z* 363.1919









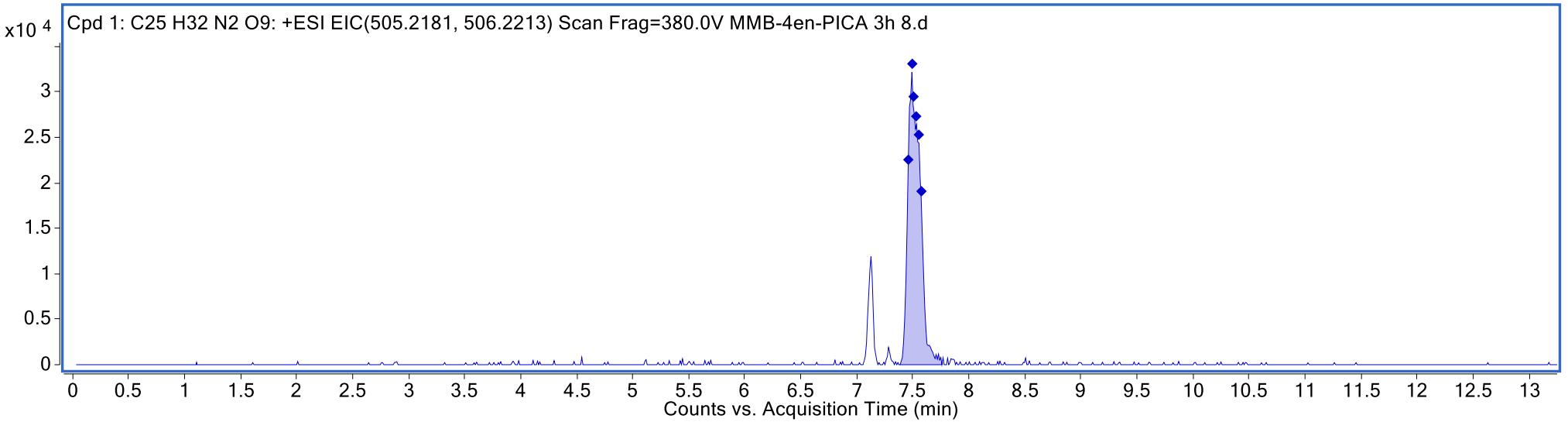


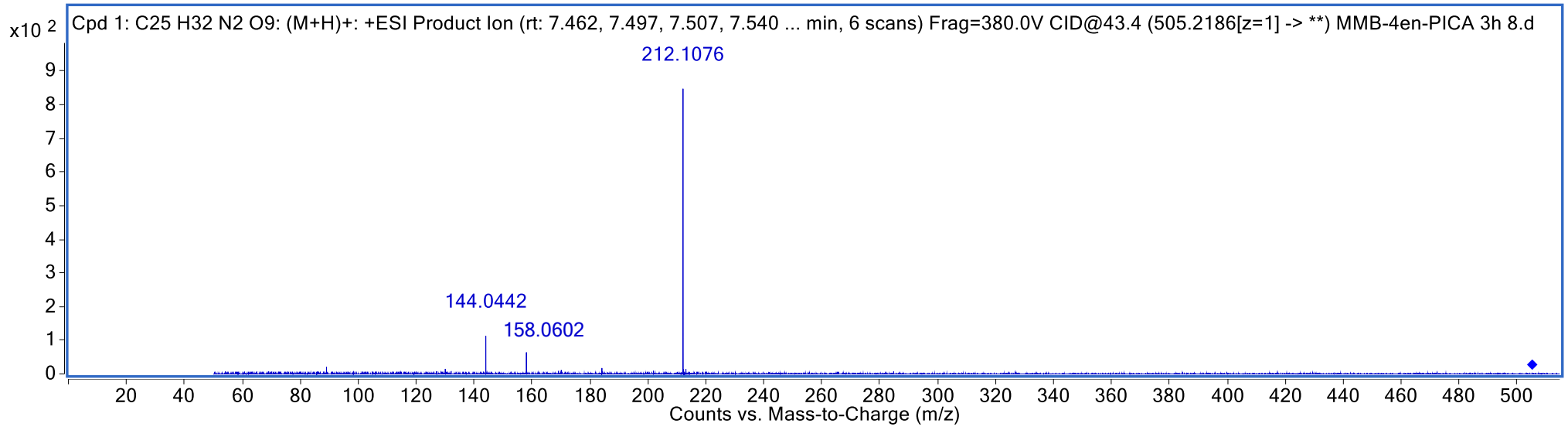
$-H_2O$

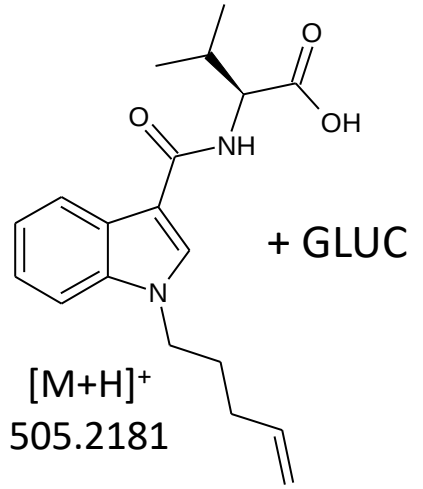
85.0648 → 67.0542

$-H_2O$

A3, Ester hydrolysis + glucuronidation, RT 7.49 min, *m/z* 505.2180

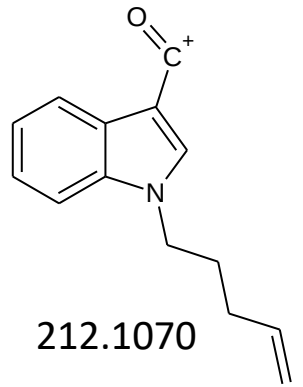






+ GLUC

$[M+H]^+$
505.2181



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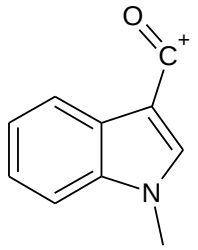

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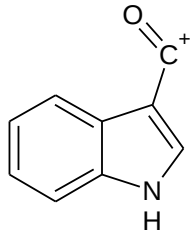
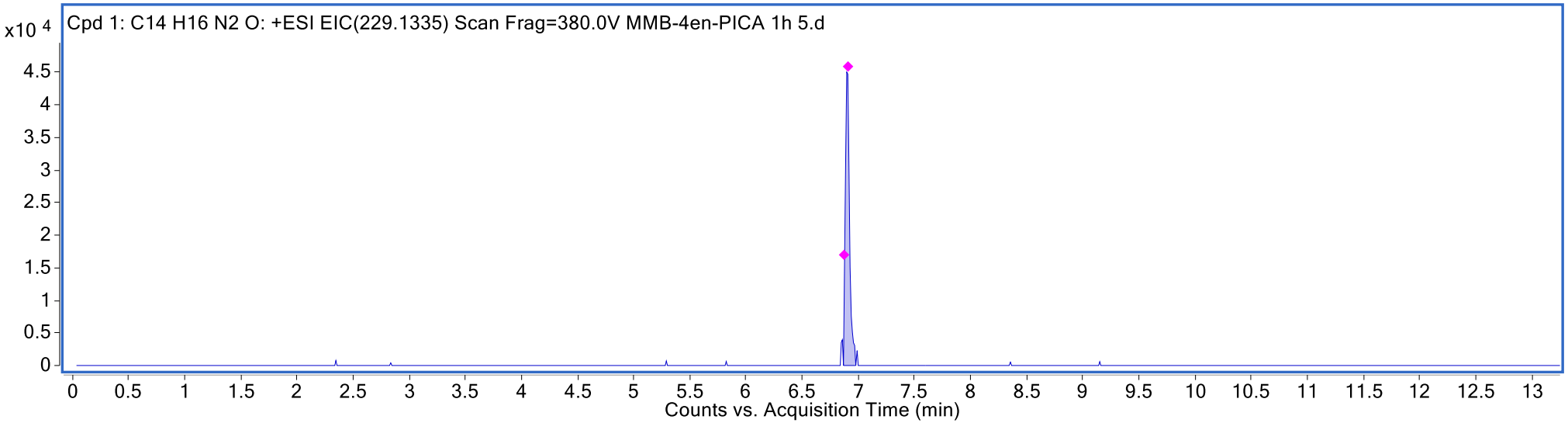
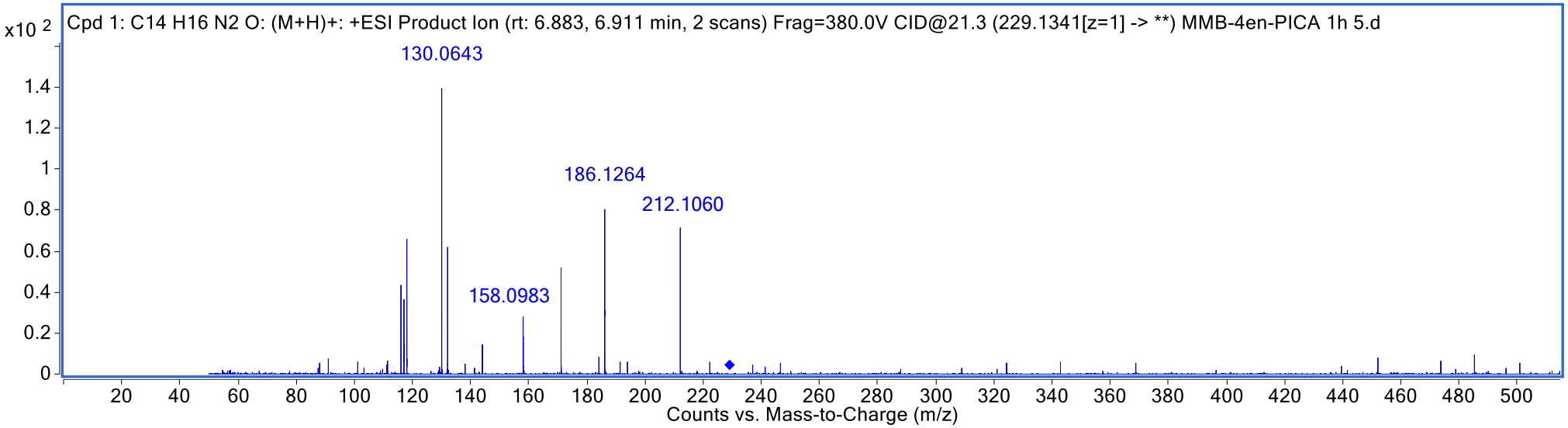
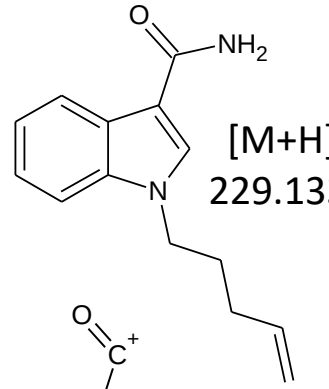


144.0444

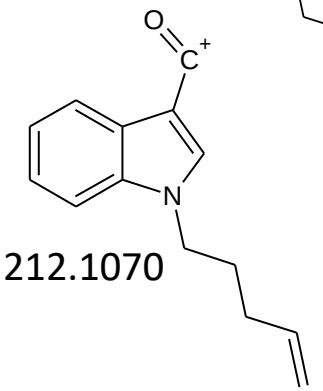
A4, Head group–linker cleavage, RT 6.91 min, *m/z* 229.1340







[M+H]+
229.1335



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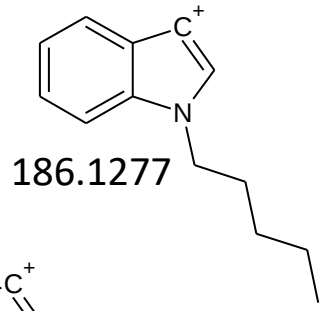
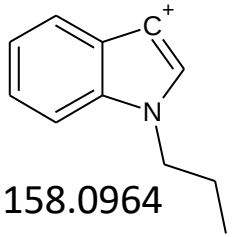


186.1277



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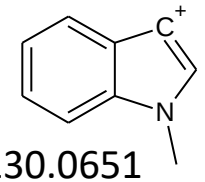
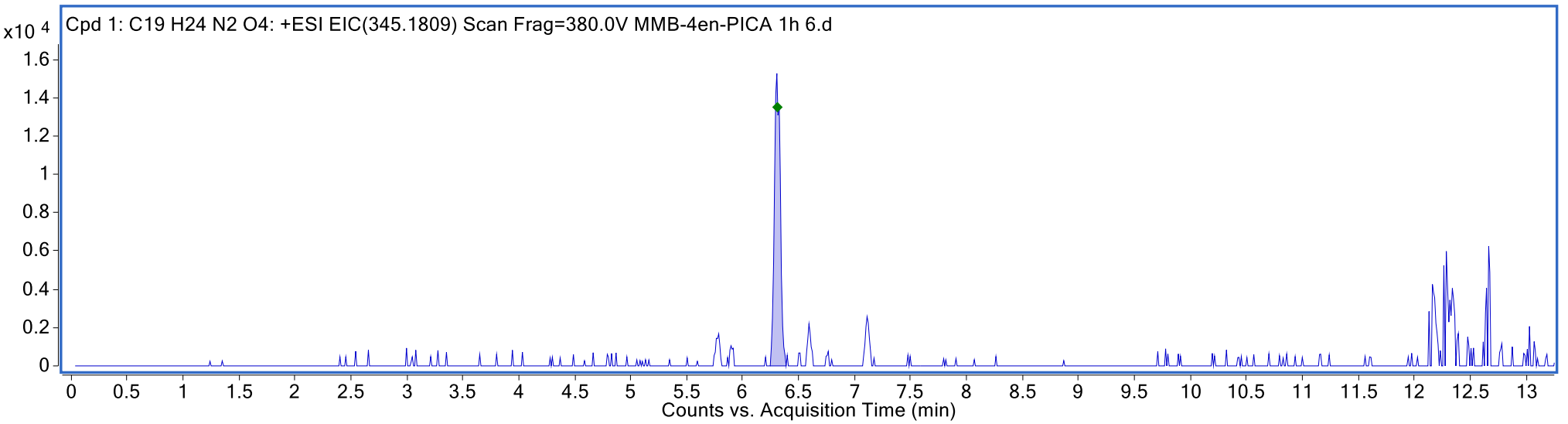
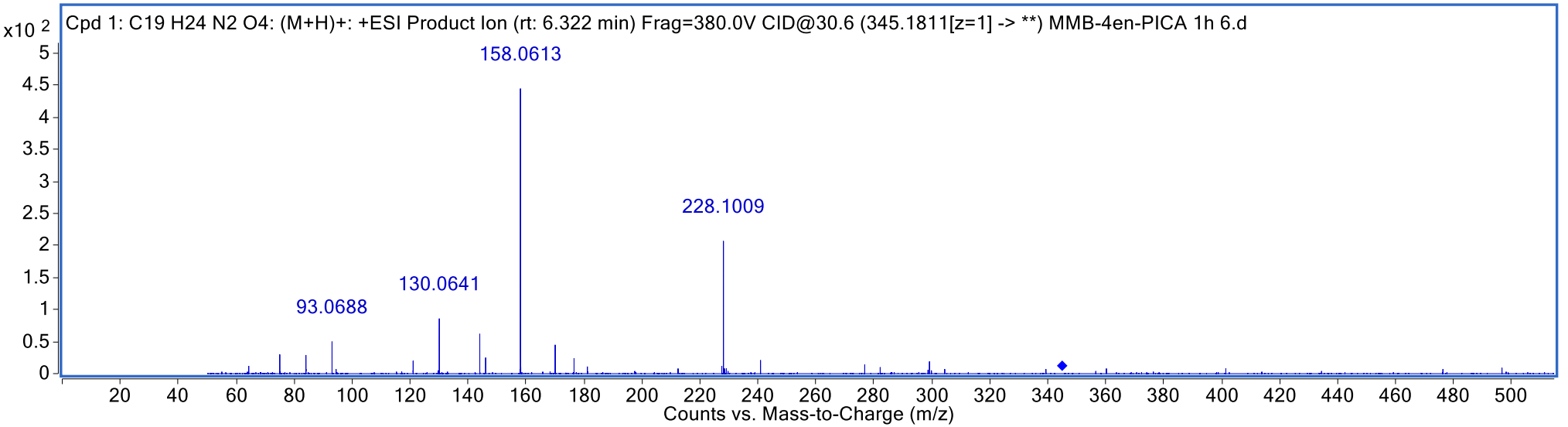
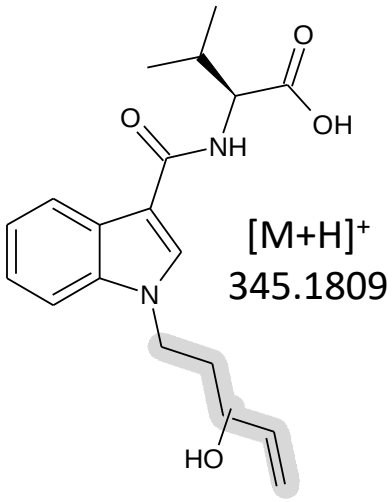


130.0651

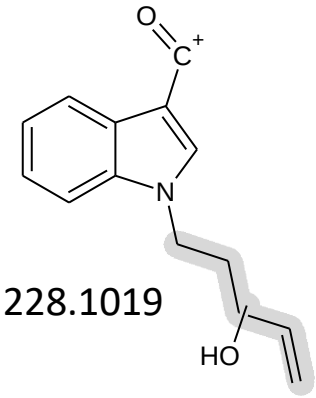
A5, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 6.31 min, *m/z* 345.1811



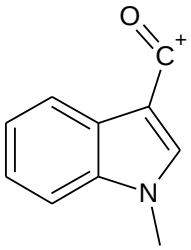




$[M+H]^+$
345.1809



228.1019



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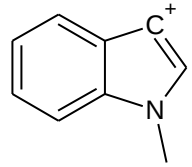
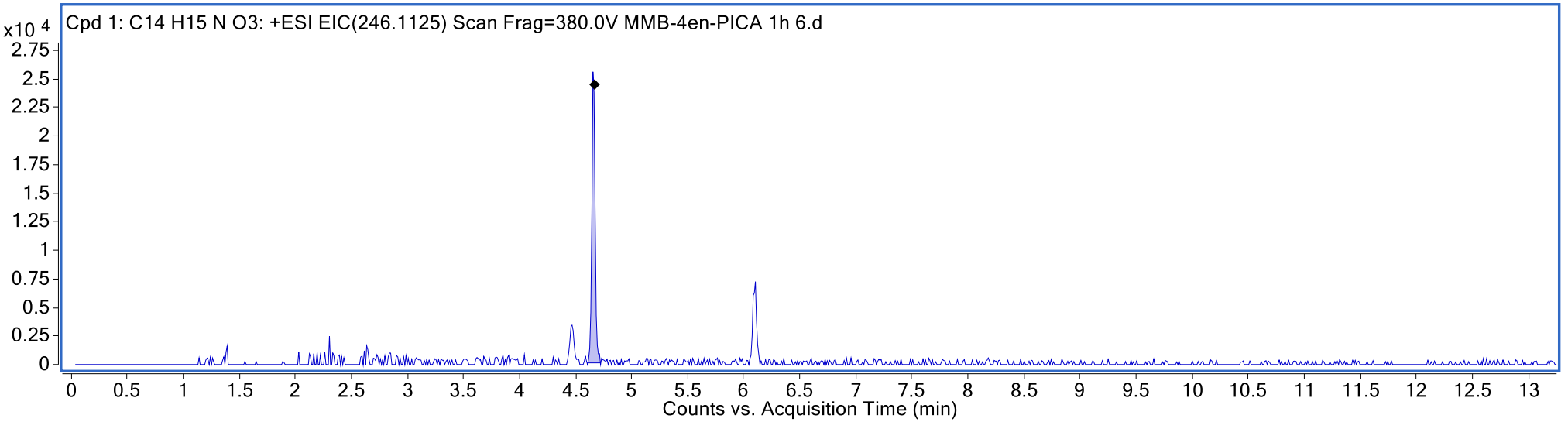
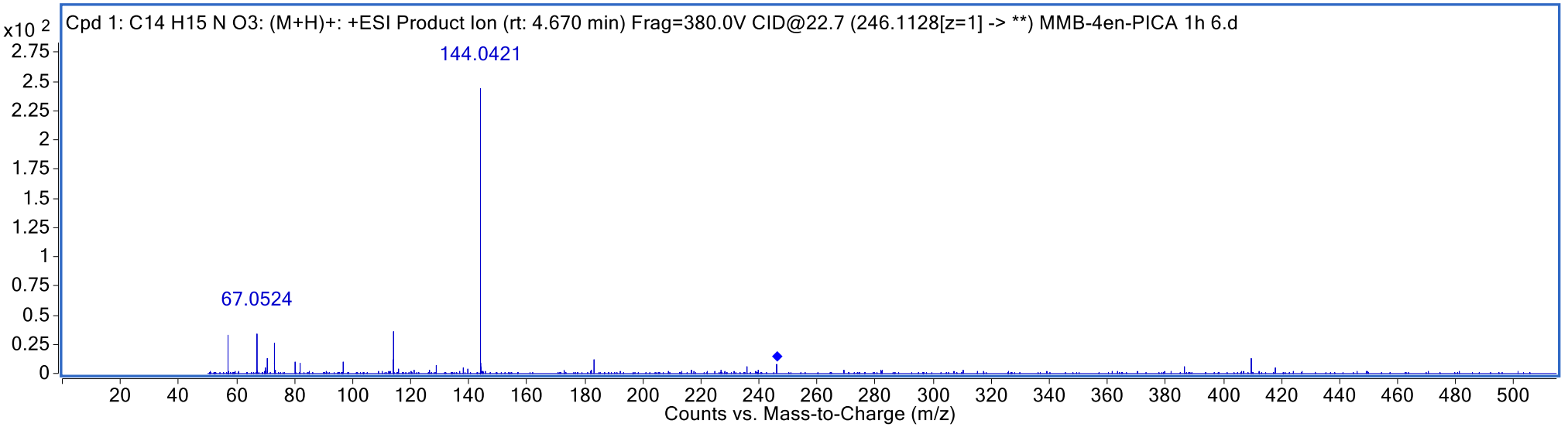
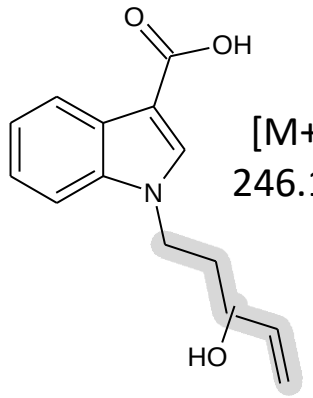


130.0651

A6, Secondary amide hydrolysis + mono-hydroxylation (pentenyl tail), RT 4.66 min, *m/z* 246.1129







$[M+H]^+$
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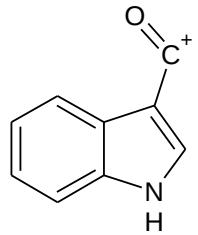

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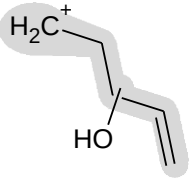
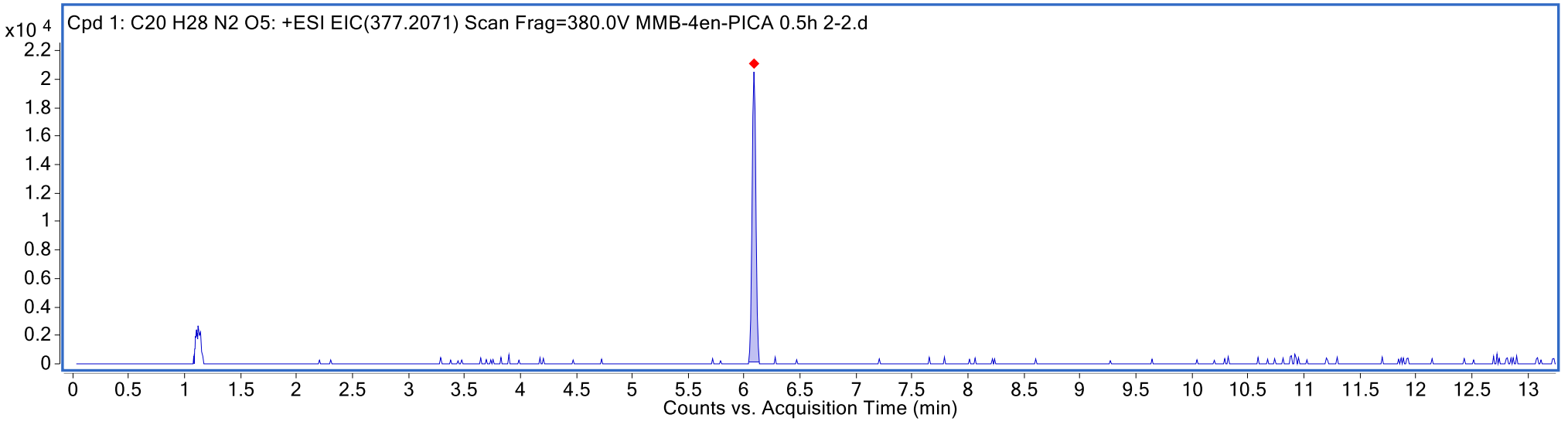
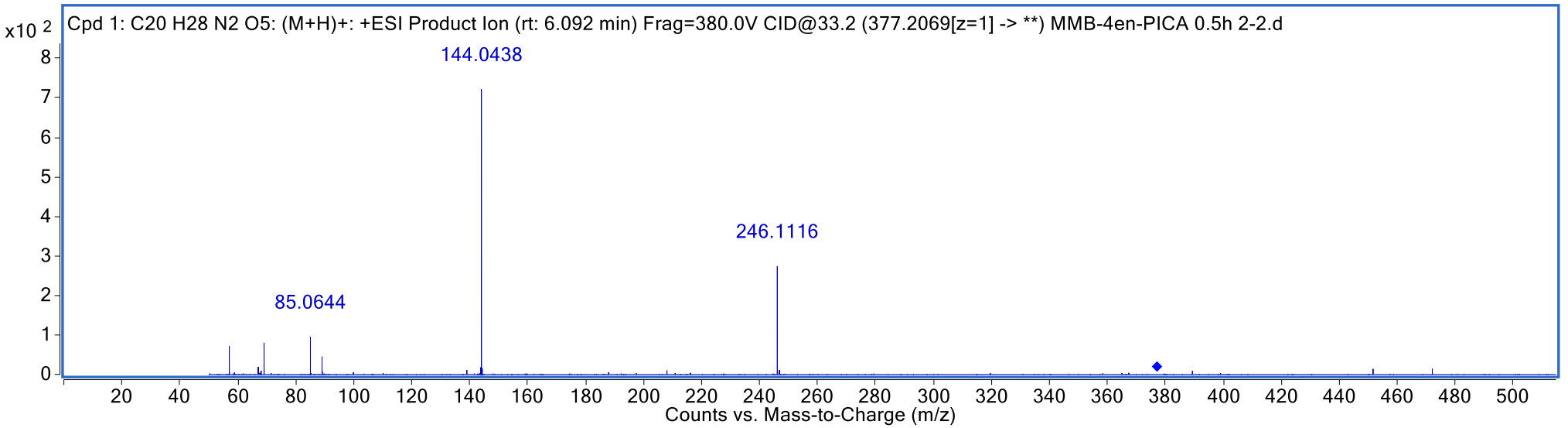
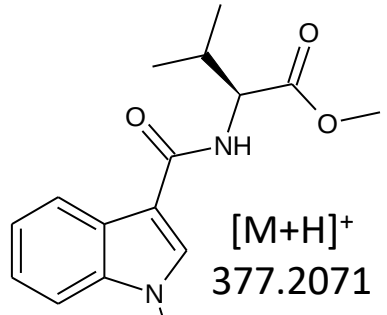


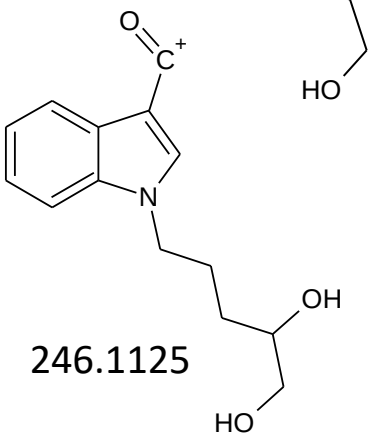
85.0648 (not detected) $\xrightarrow{-H_2O}$ 67.0542

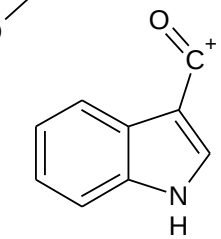
A7, Dihydrodiol formation, RT 6.09 min, *m/z* 377.2069

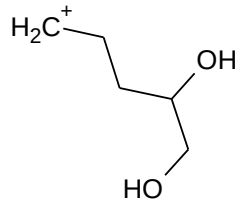




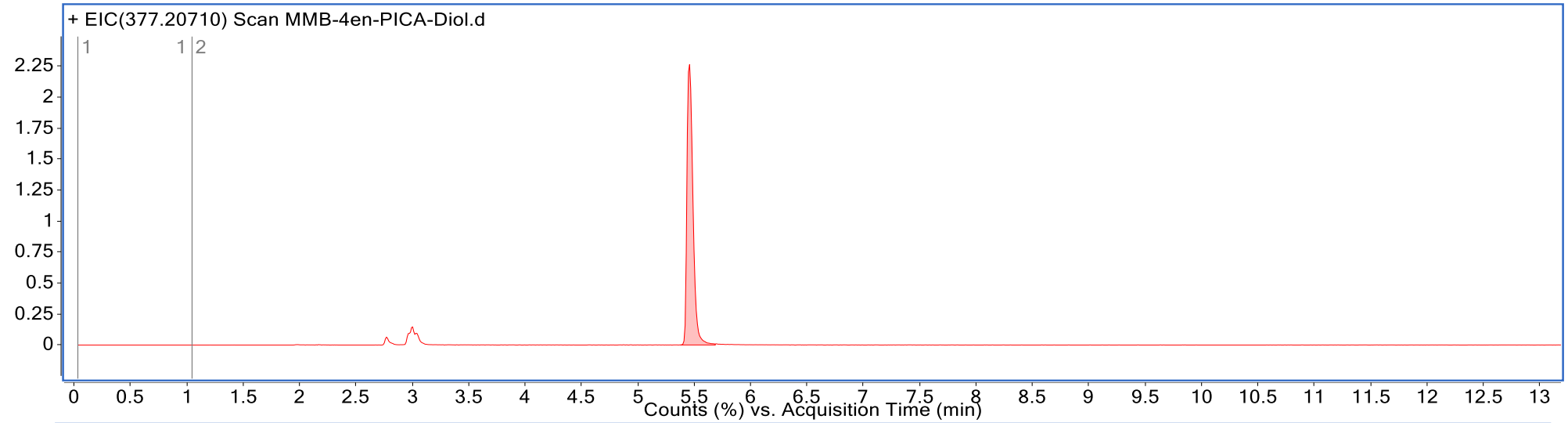


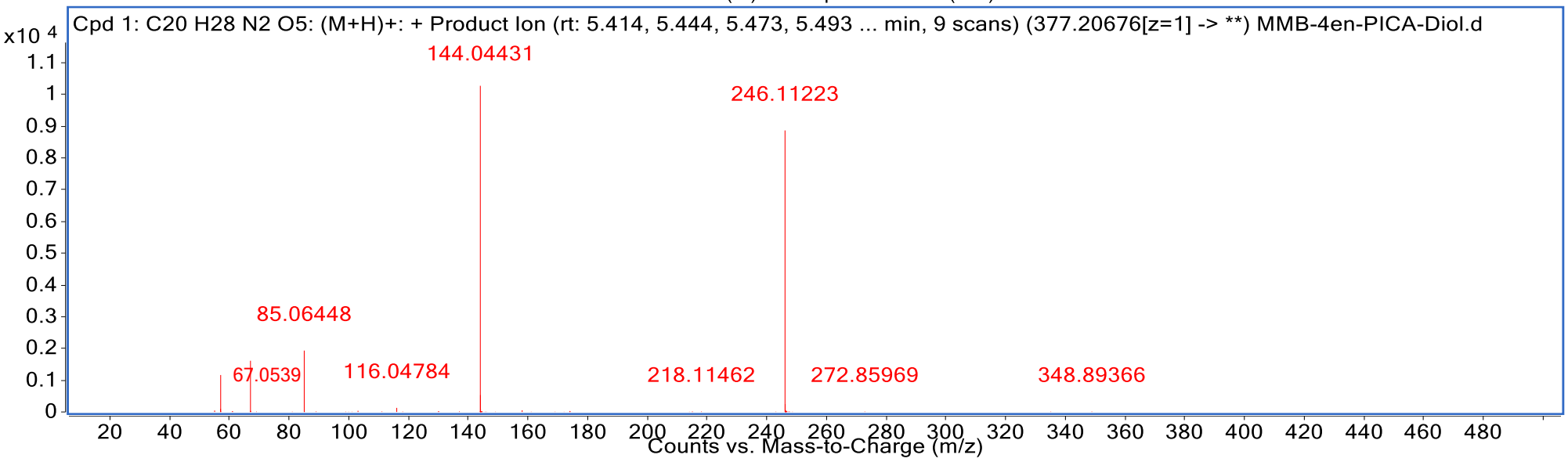


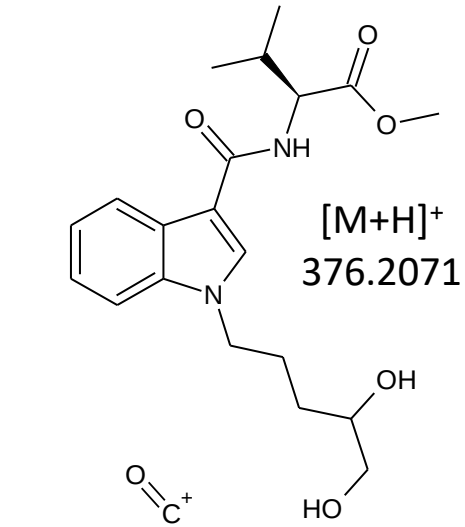


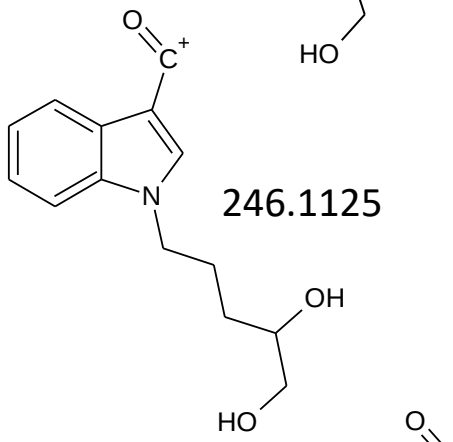


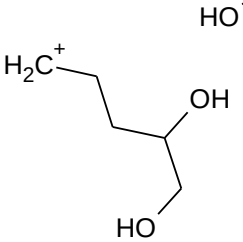
Dihydrodiol reference standard, RT 5.46 min, *m/z* 377.2067

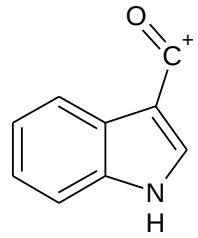












$-H_2O$

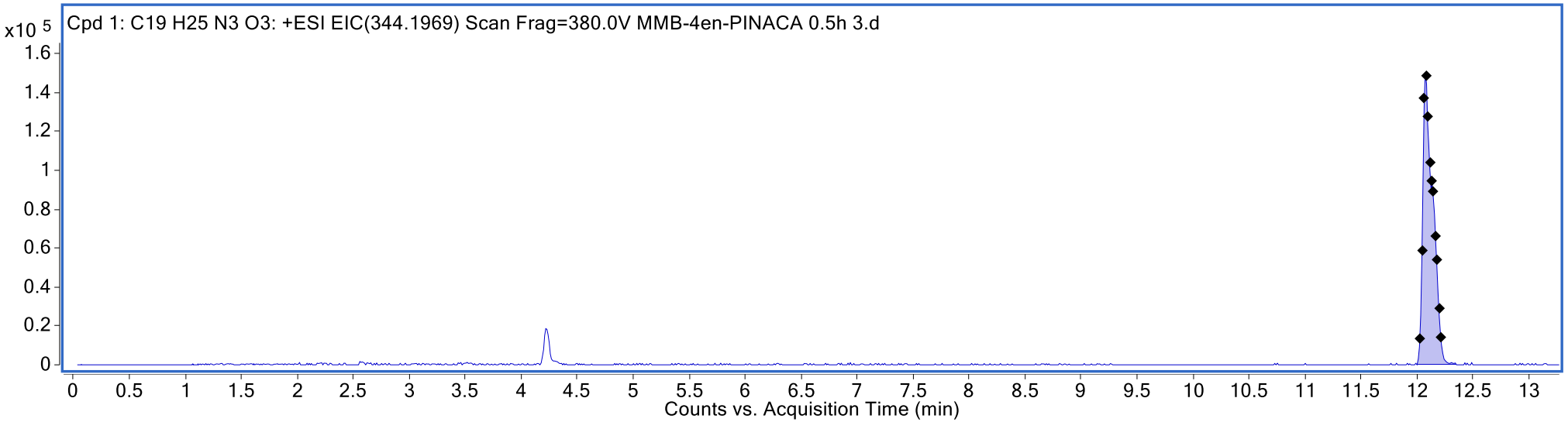
85.0648 → 67.0542

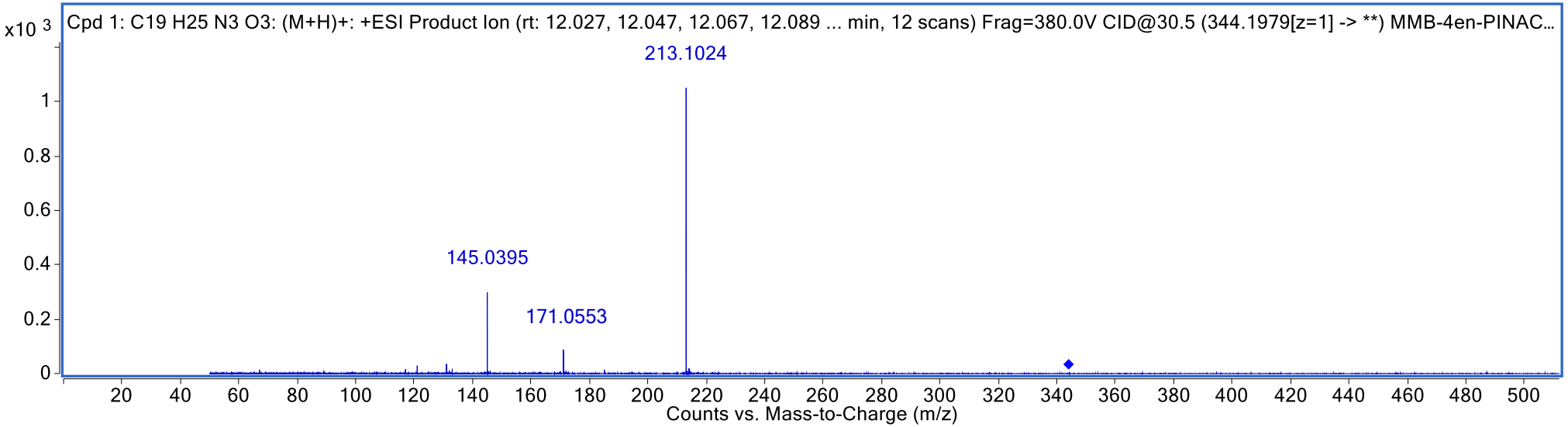
$-H_2O$

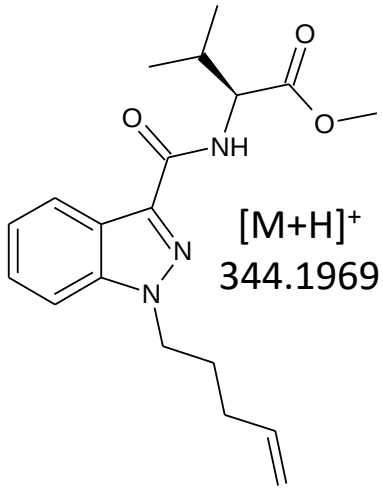
MMB-4en-PINACA

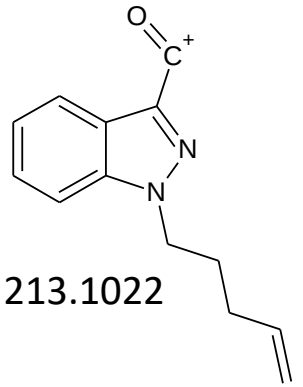
Metabolism

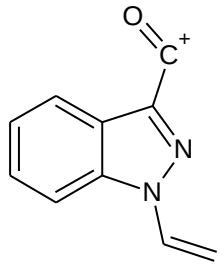
MMB-4en-PINACA, RT 12.05 min, *m/z* 344.1989

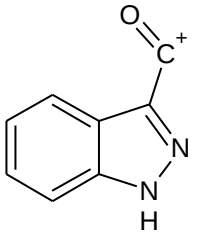




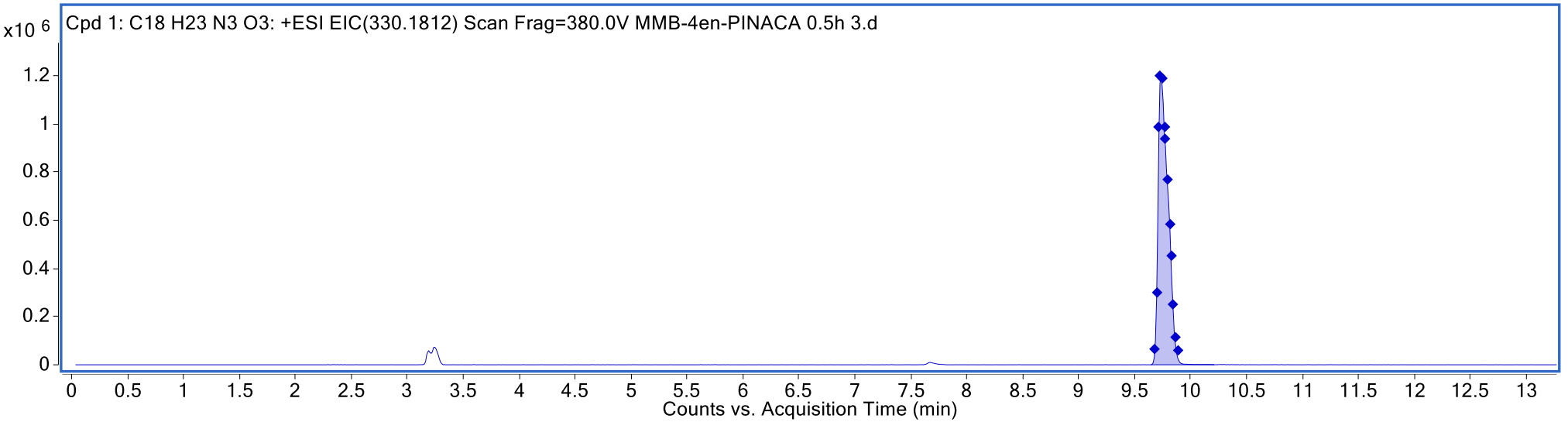


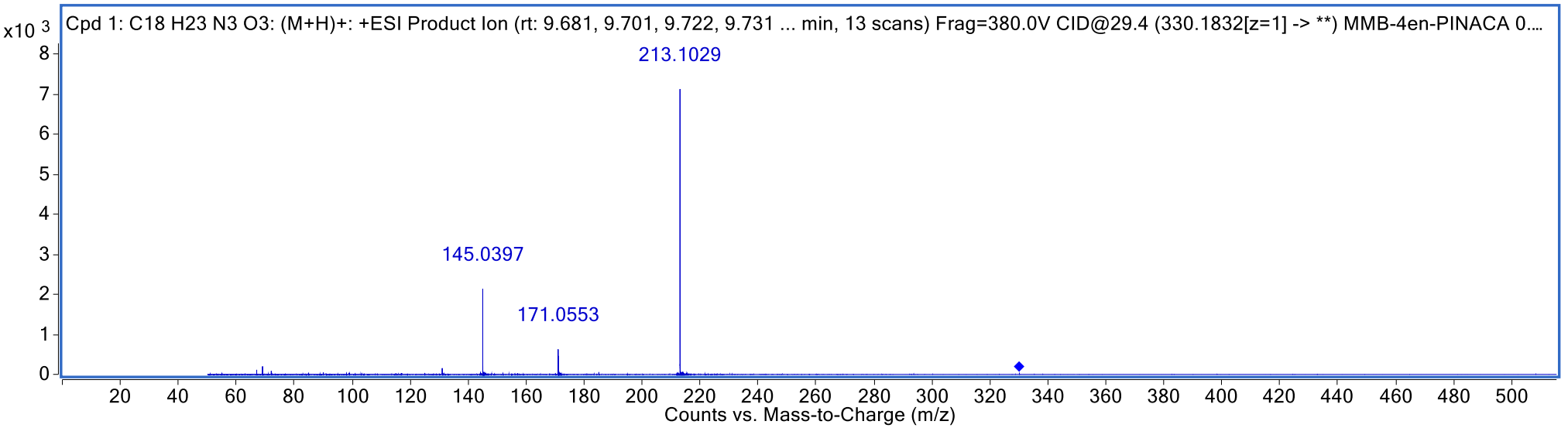


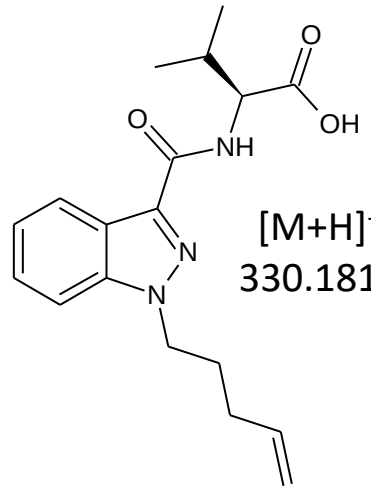




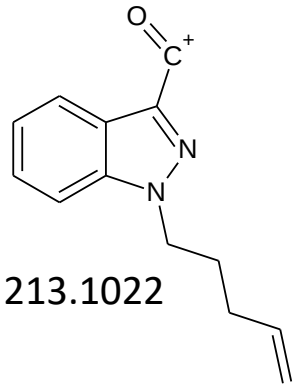
B1, Ester hydrolysis, RT 9.72 min, *m/z* 330.1827



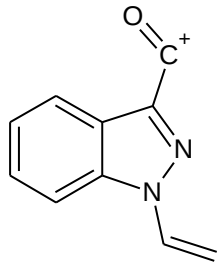




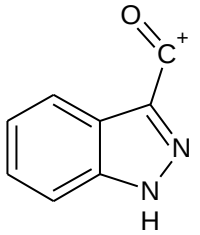
[M+H]+
330.1812



213.1022

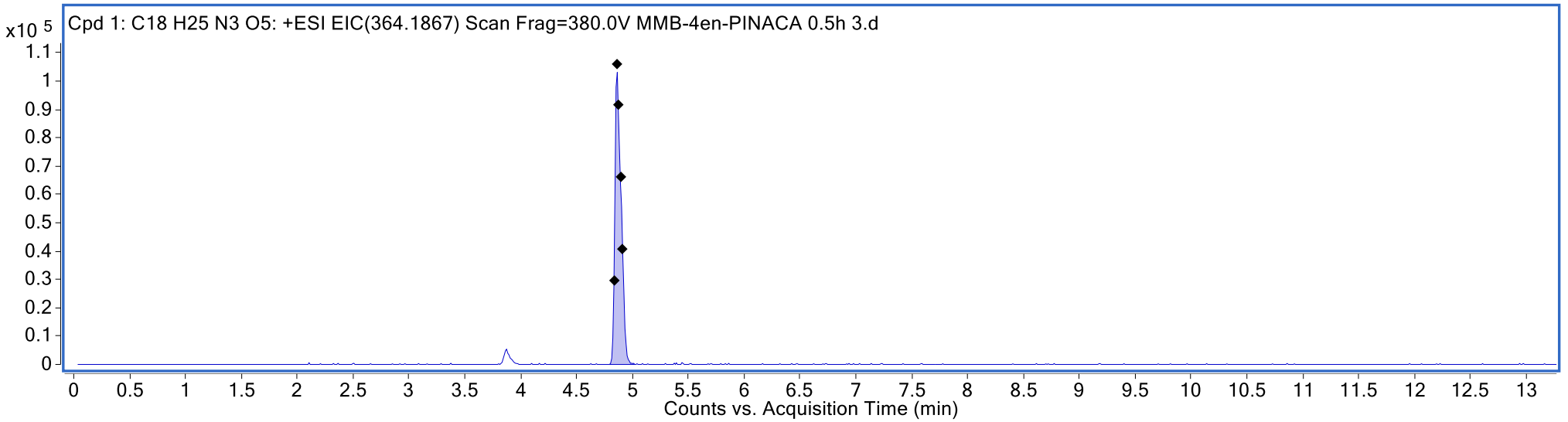


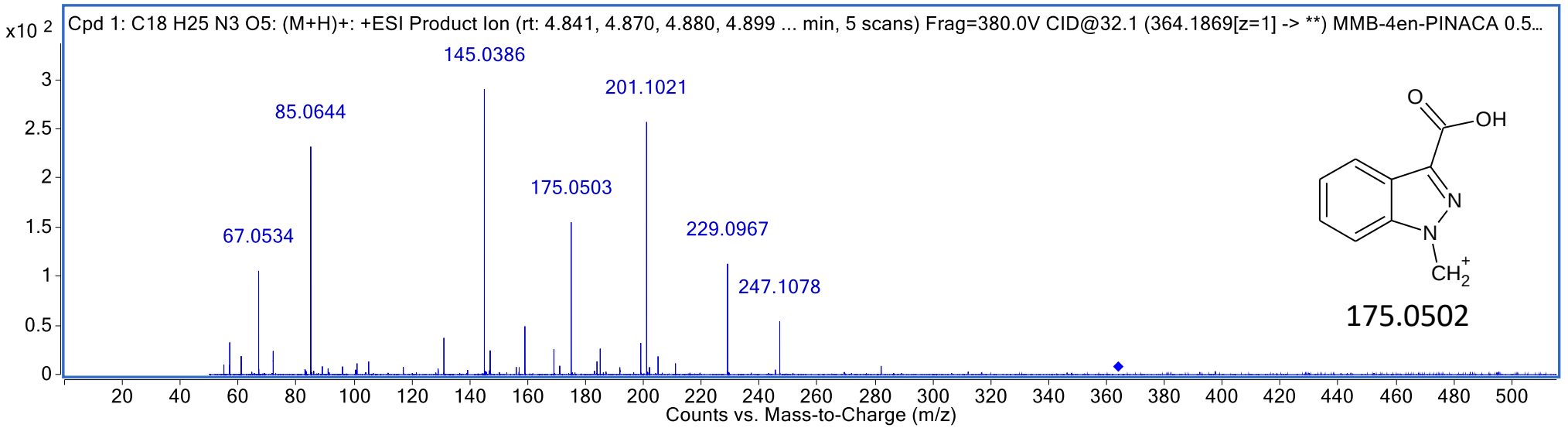
171.0553

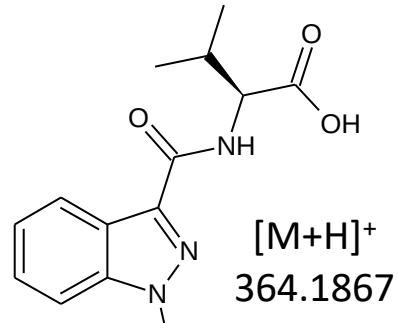


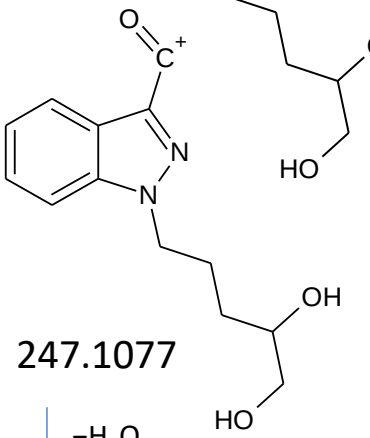
145.0396

B2, Ester hydrolysis + dihydrodiol formation, RT 4.85 min, *m/z* 364.1873





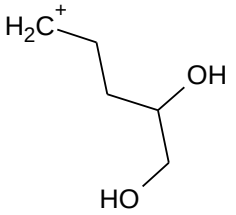


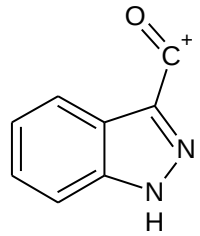


−H_2O

229.0972 → 201.1022

−CO



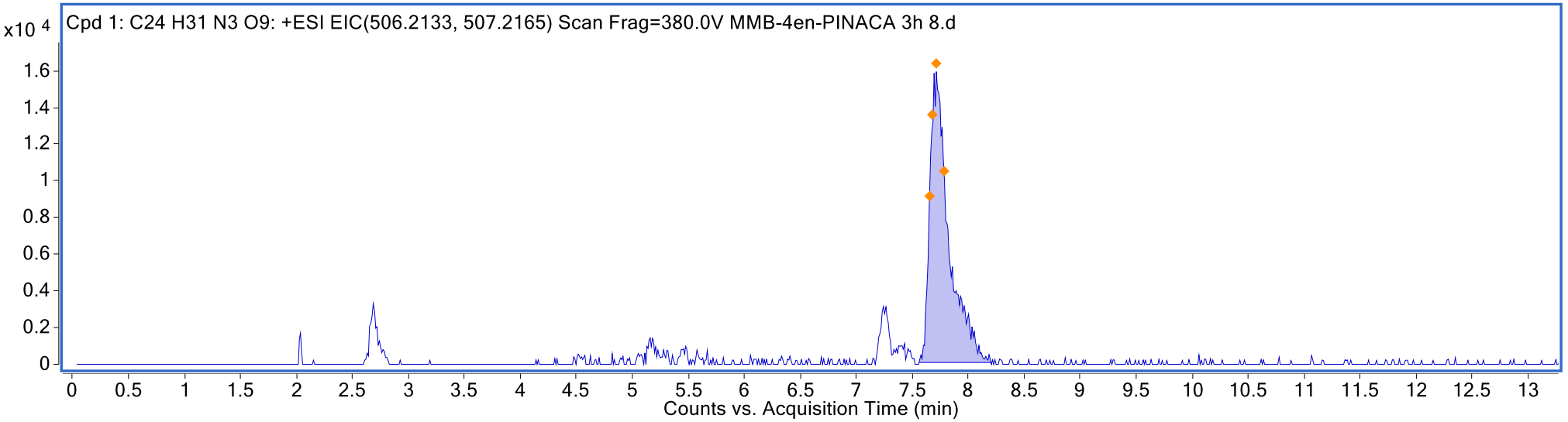


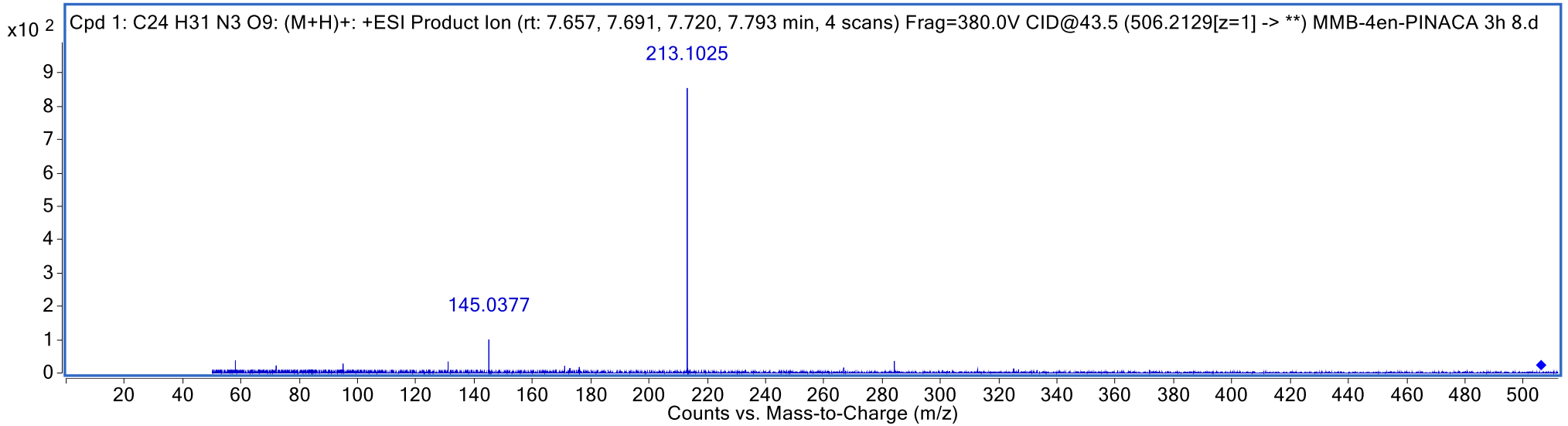
−H_2O

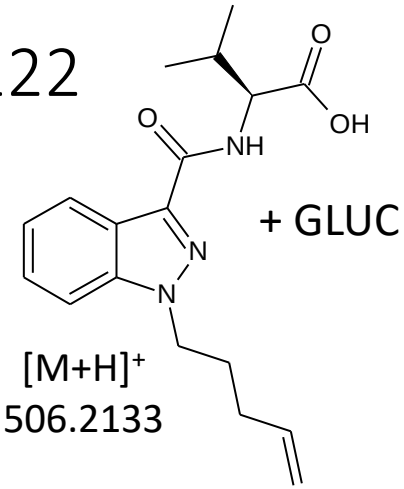
85.0648 → 67.0542

−H_2O

B3, Ester hydrolysis + glucuronidation, RT 7.68 min, *m/z* 506.2122

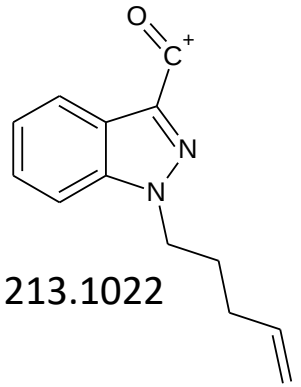




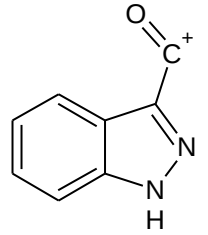


+ GLUC

$[M+H]^+$
506.2133

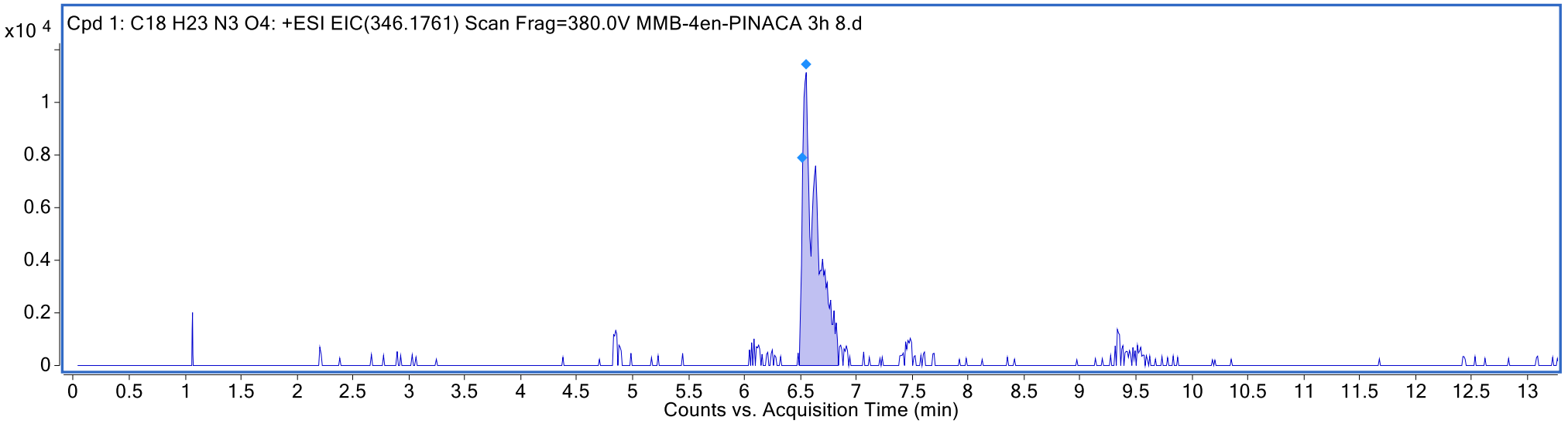


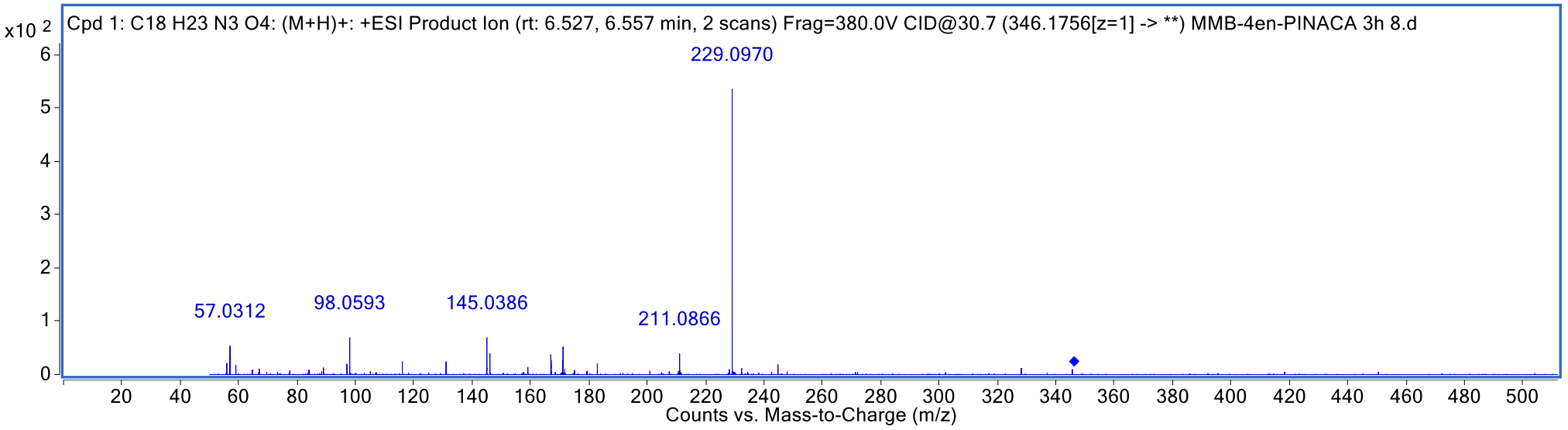
213.1022

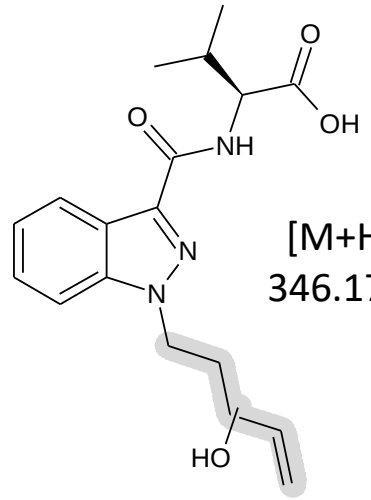


145.0396

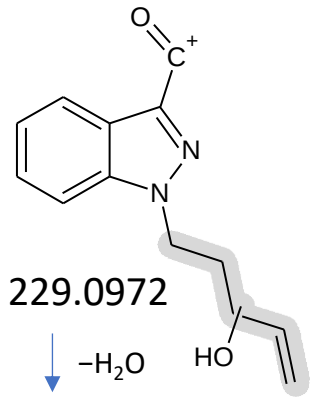
B4, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 6.55 min, *m/z* 346.1756







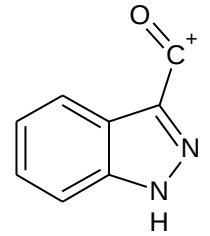
$[M+H]^+$
346.1761



229.0972

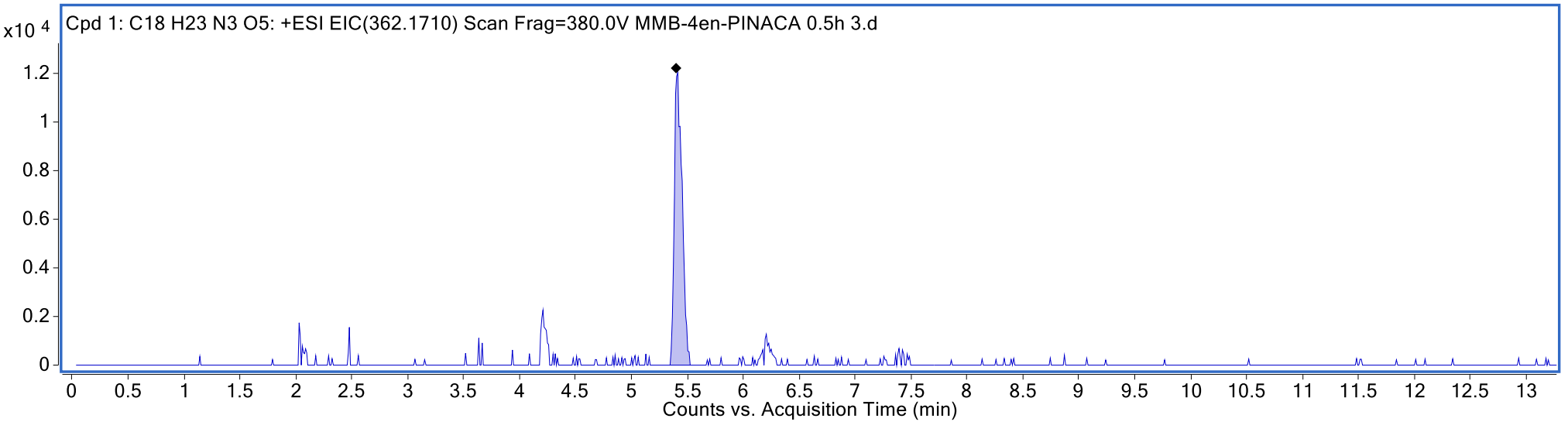
$-H_2O$

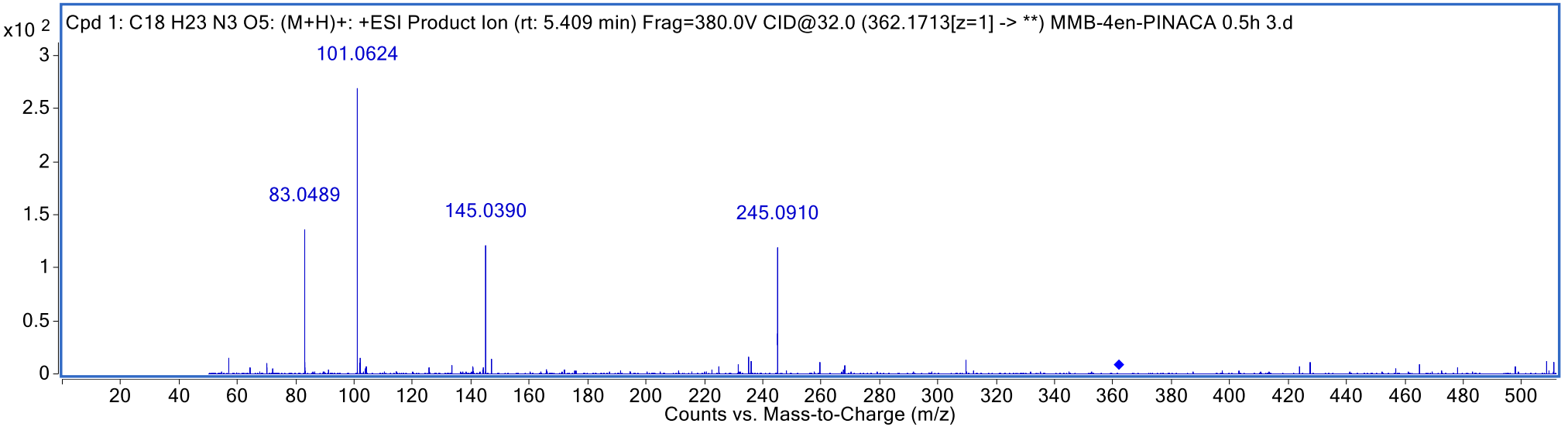
211.0866

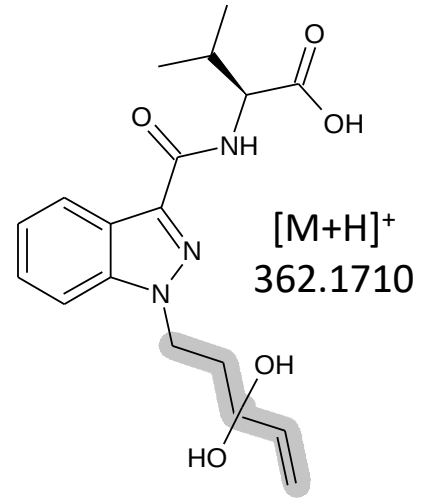


145.0396

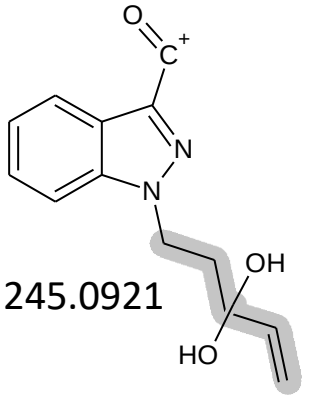
B5, Ester hydrolysis + di-hydroxylation (pentenyl tail), RT 5.39 min, *m/z* 362.1709



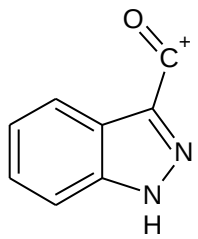




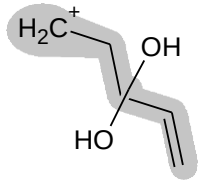
[M+H]+
362.1710



245.0921



145.0396

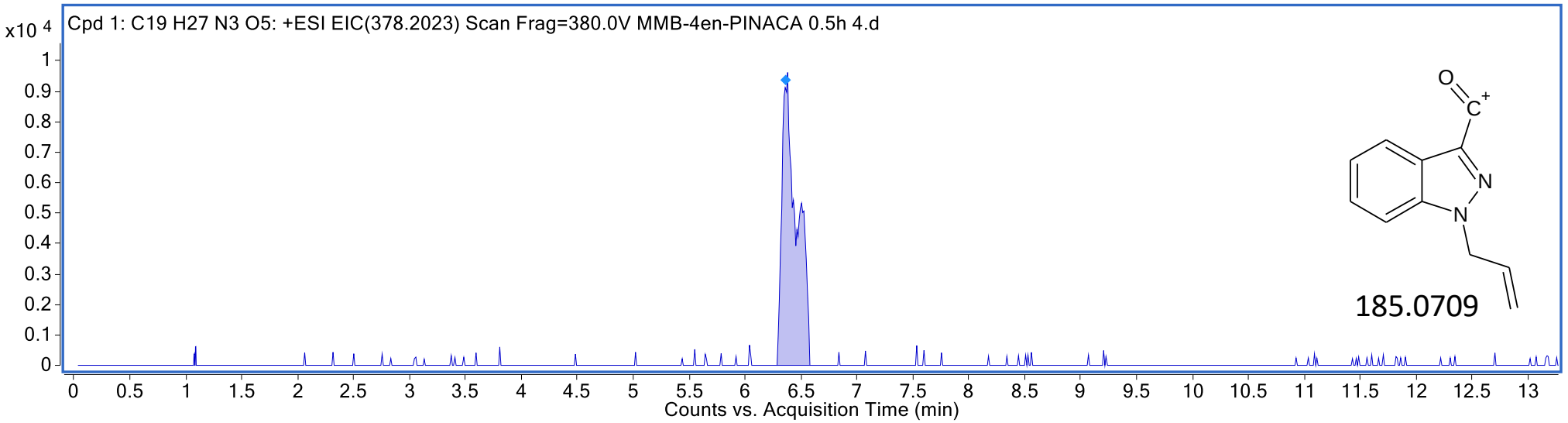


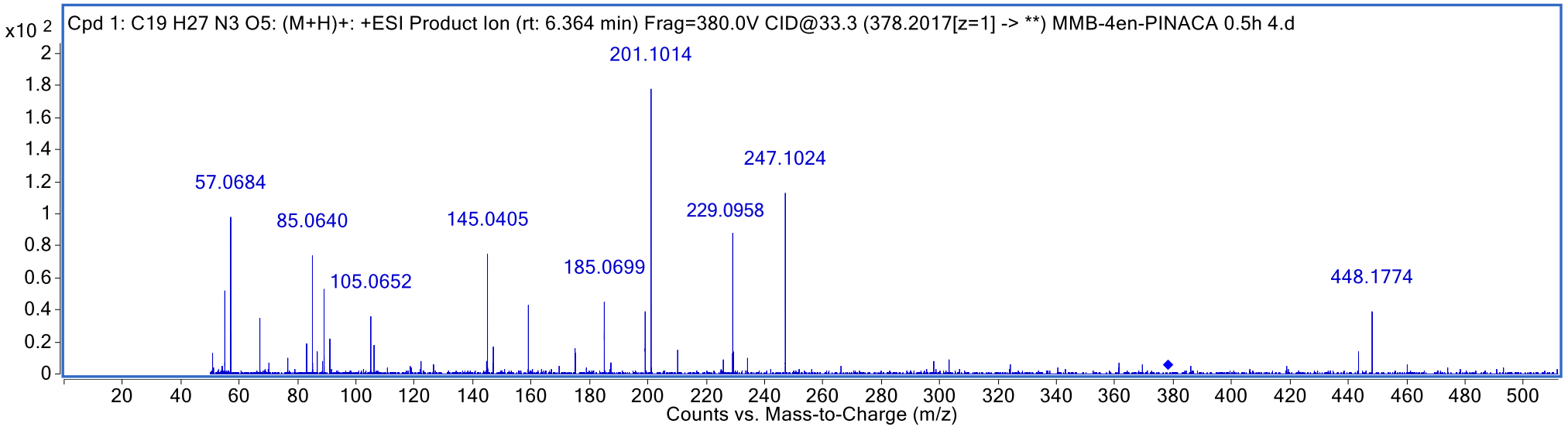
101.0597

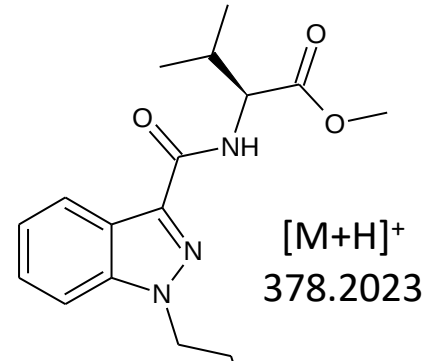
$-H_2O$

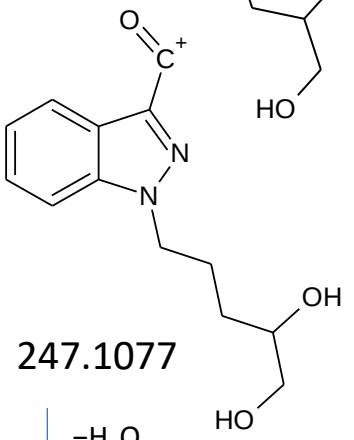
83.0491

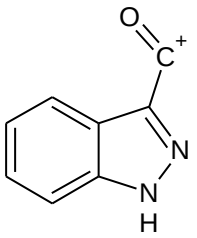
B6, Dihydrodiol formation, RT 6.36 min, *m/z* 378.2026

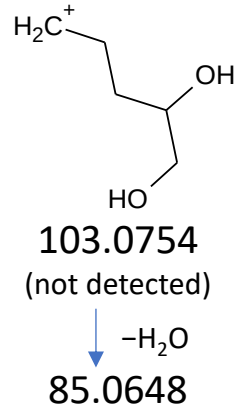




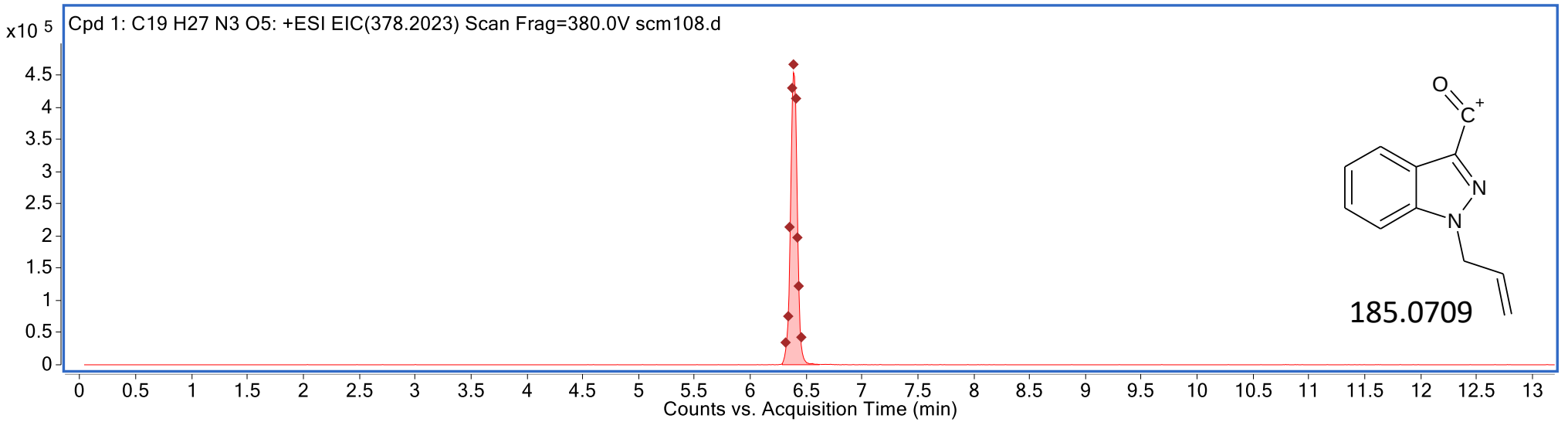


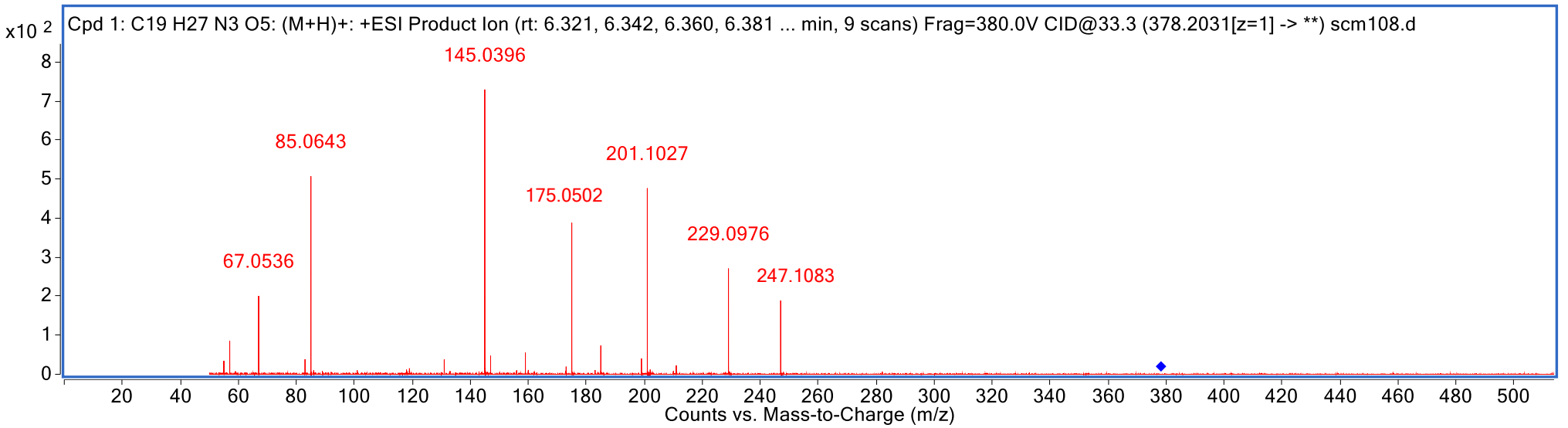


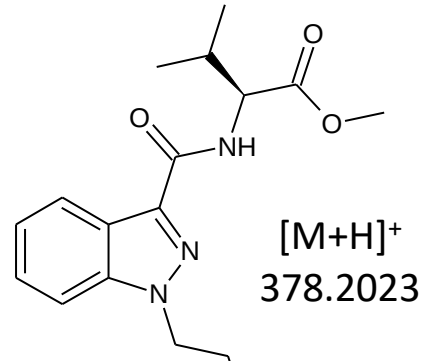


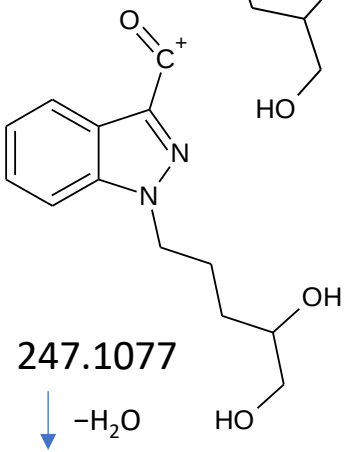


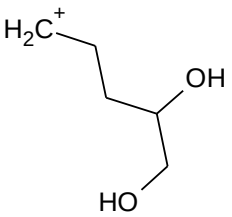
Dihydrodiol reference standard, RT 6.39 min, *m/z* 378.2031

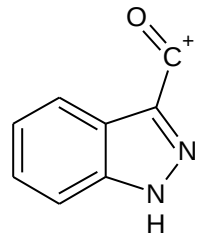








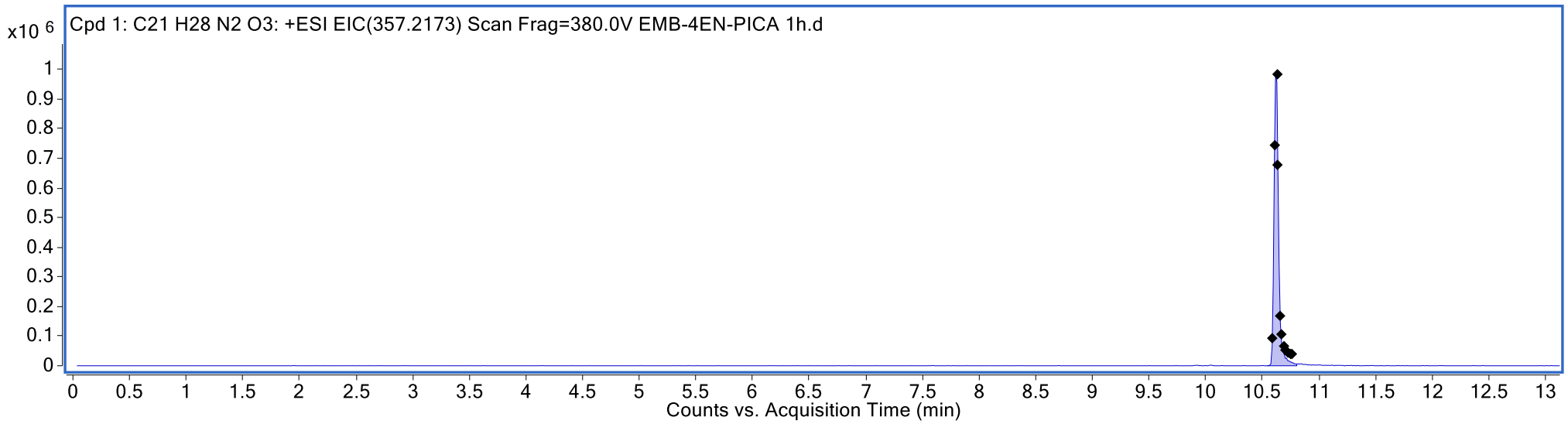


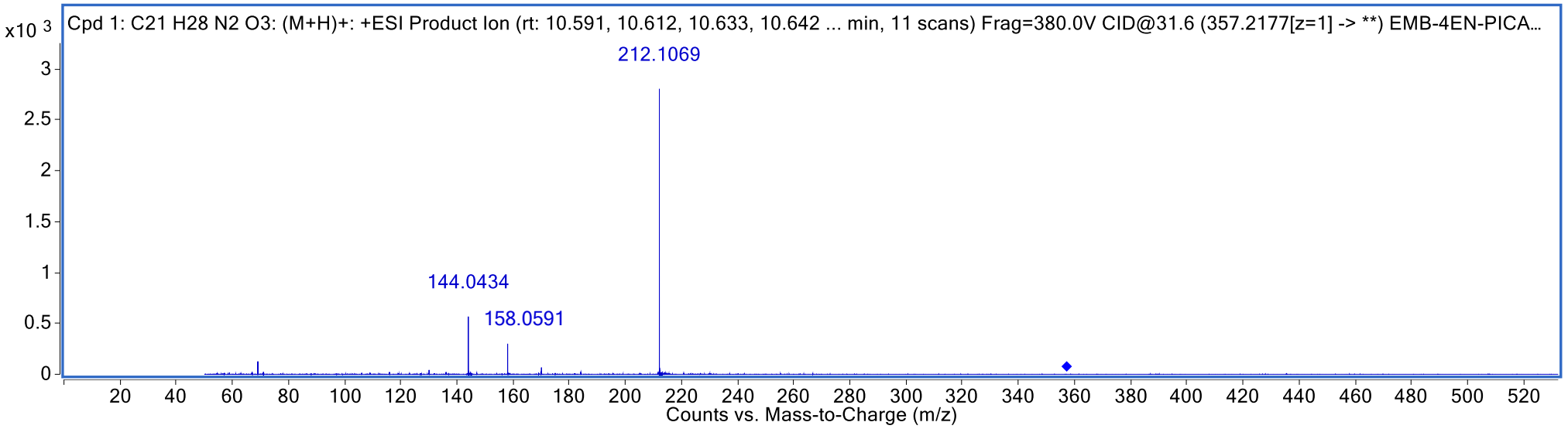


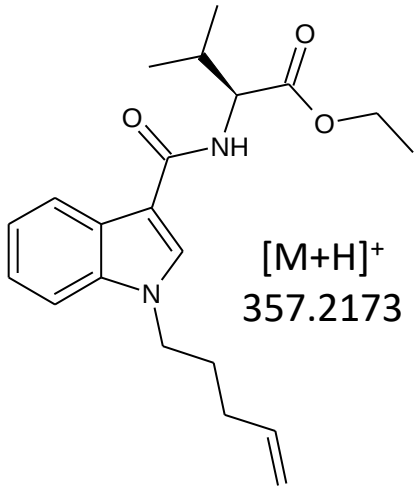
EMB-4en-PICA

Metabolism

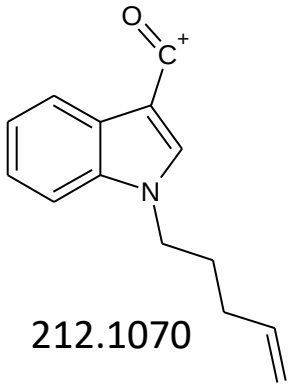
EMB-4en-PICA, RT 10.62 min, *m/z* 357.2188



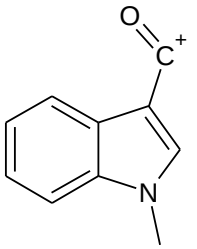




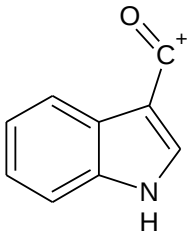
$[M+H]^+$
357.2173



212.1070

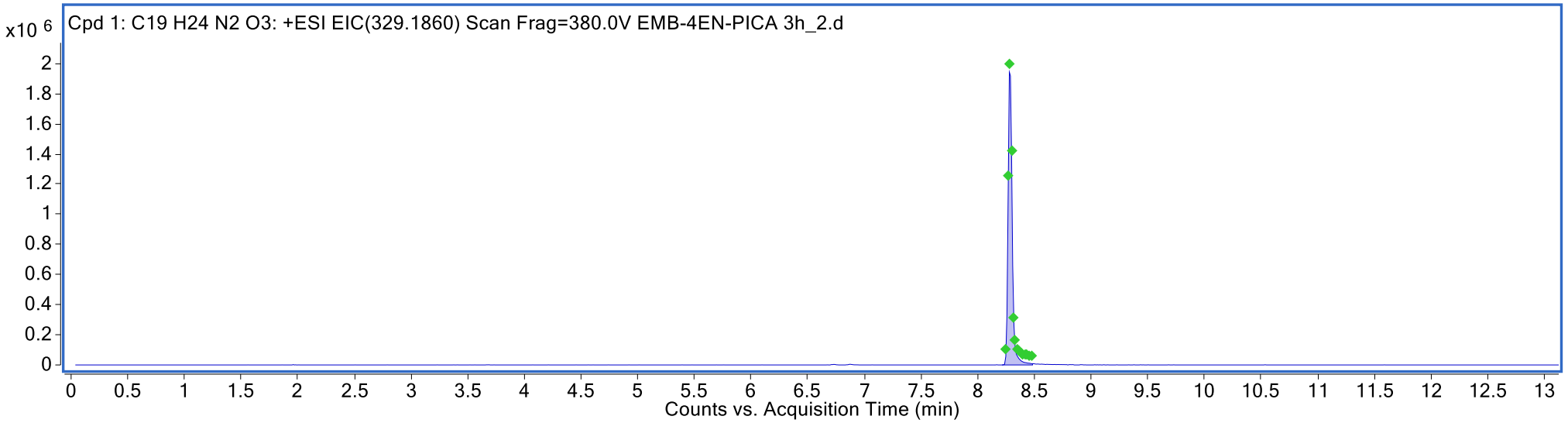


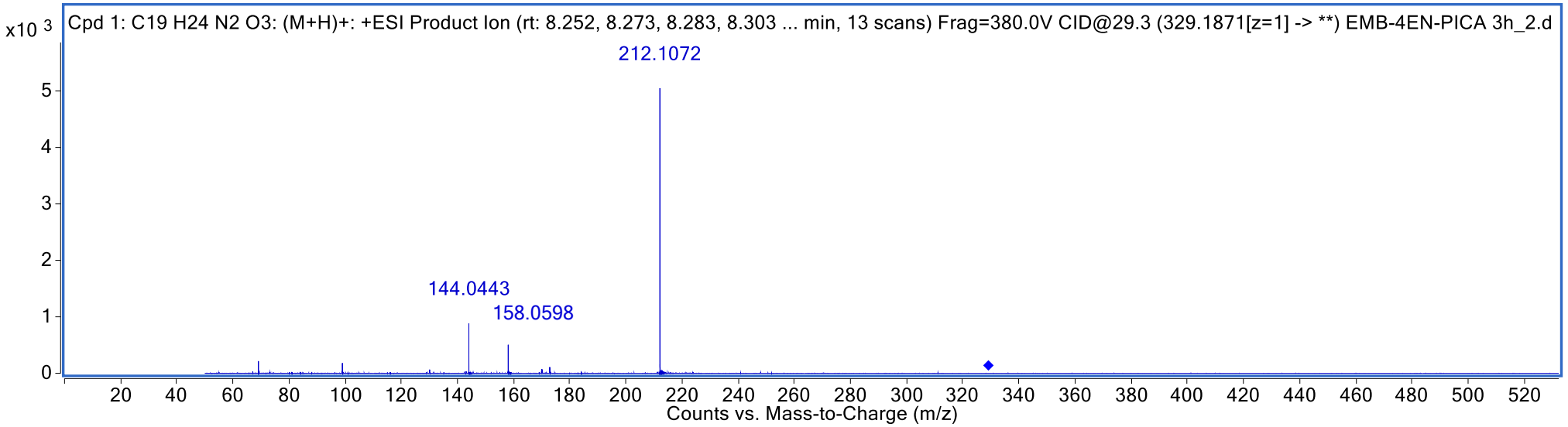
158.0600

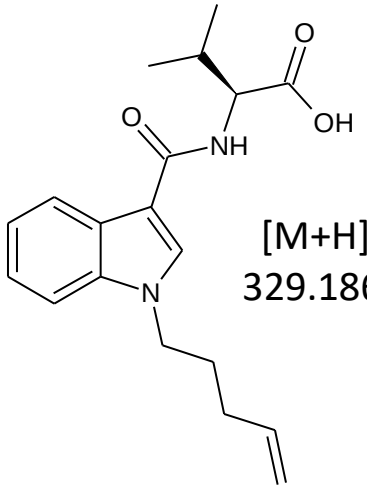


144.0444

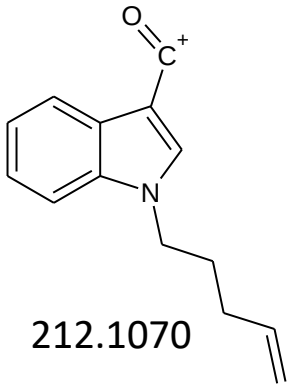
C1, Ester hydrolysis, RT 8.28 min, *m/z* 329.1868



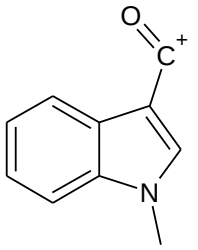




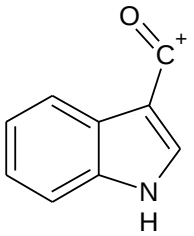
[M+H]+
329.1860



212.1070

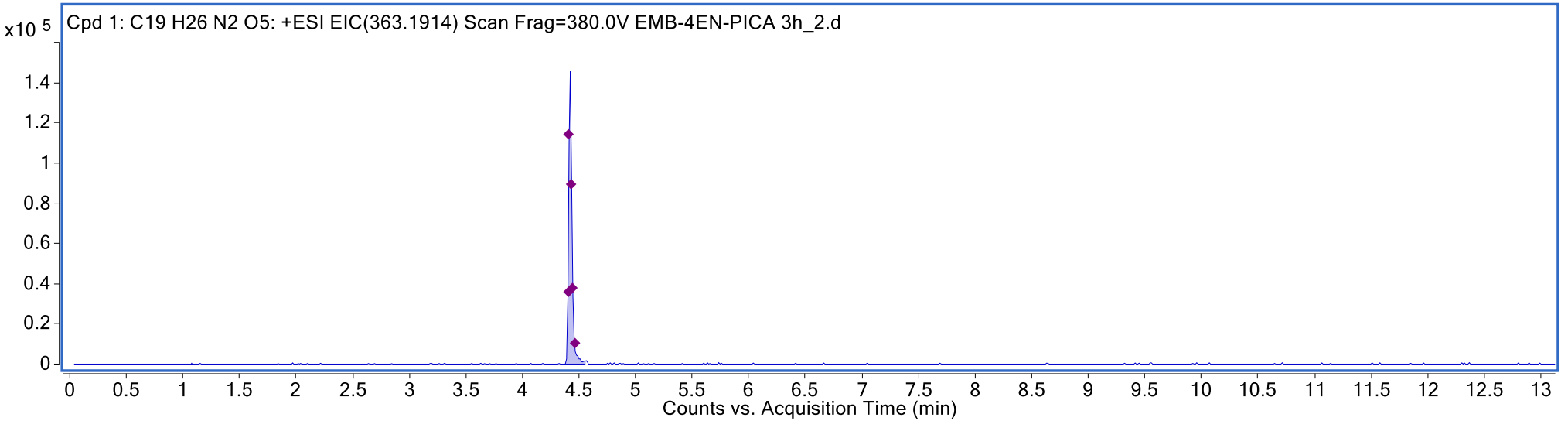


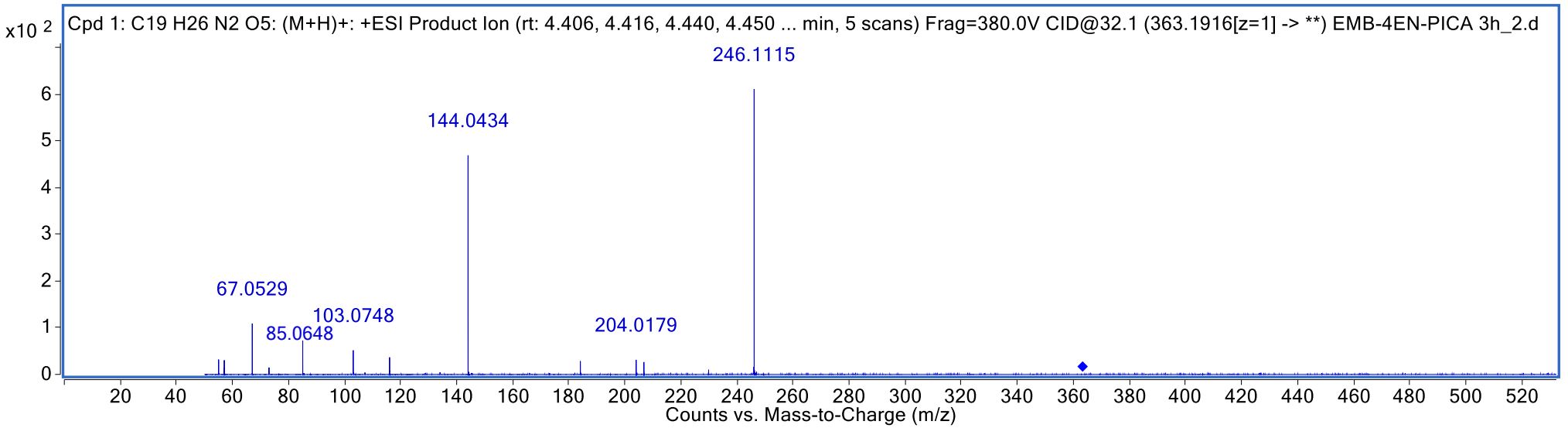
158.0600

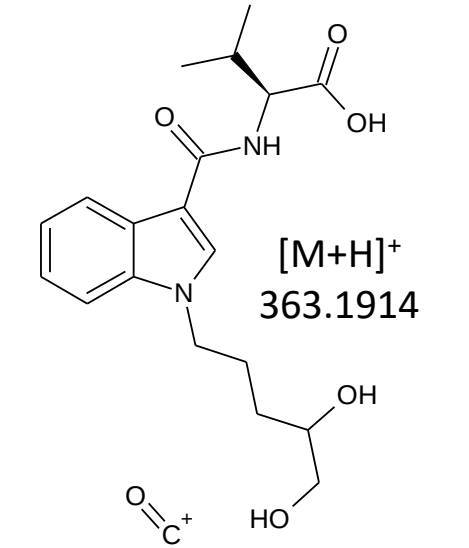


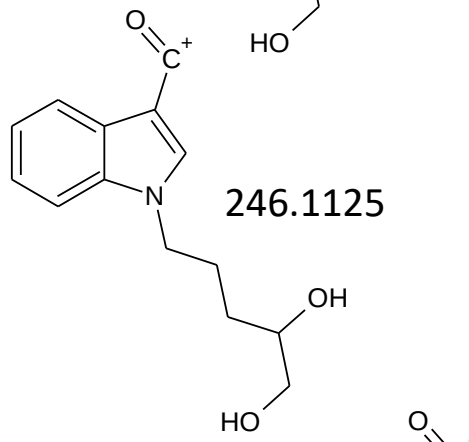
144.0444

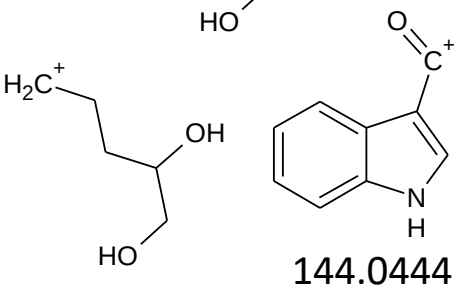
C2, Ester hydrolysis + dihydrodiol formation, RT 4.42 min, *m/z* 363.1913



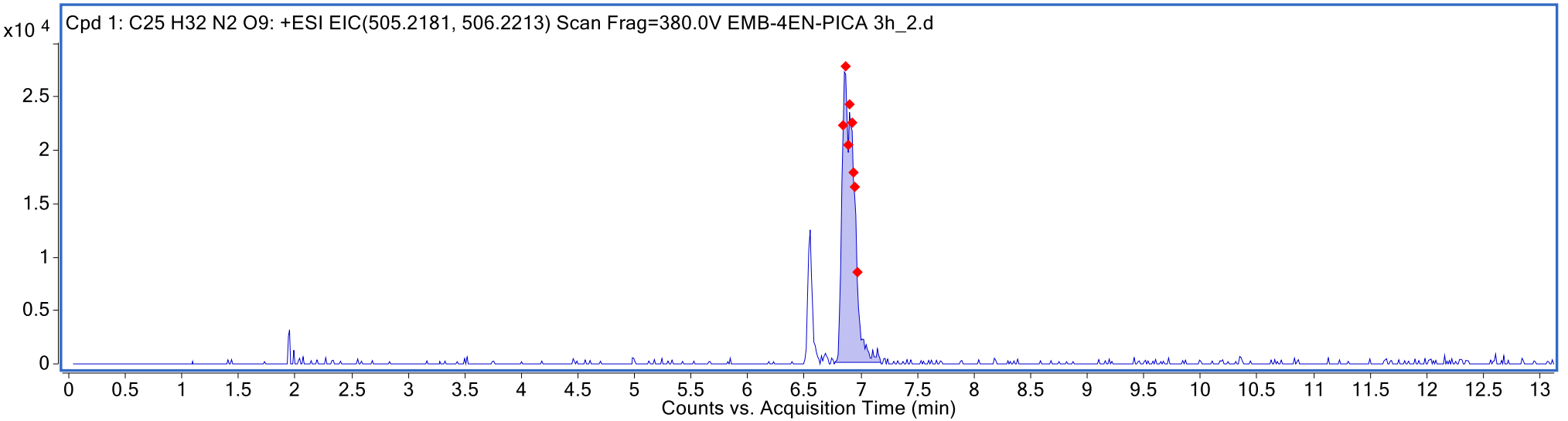


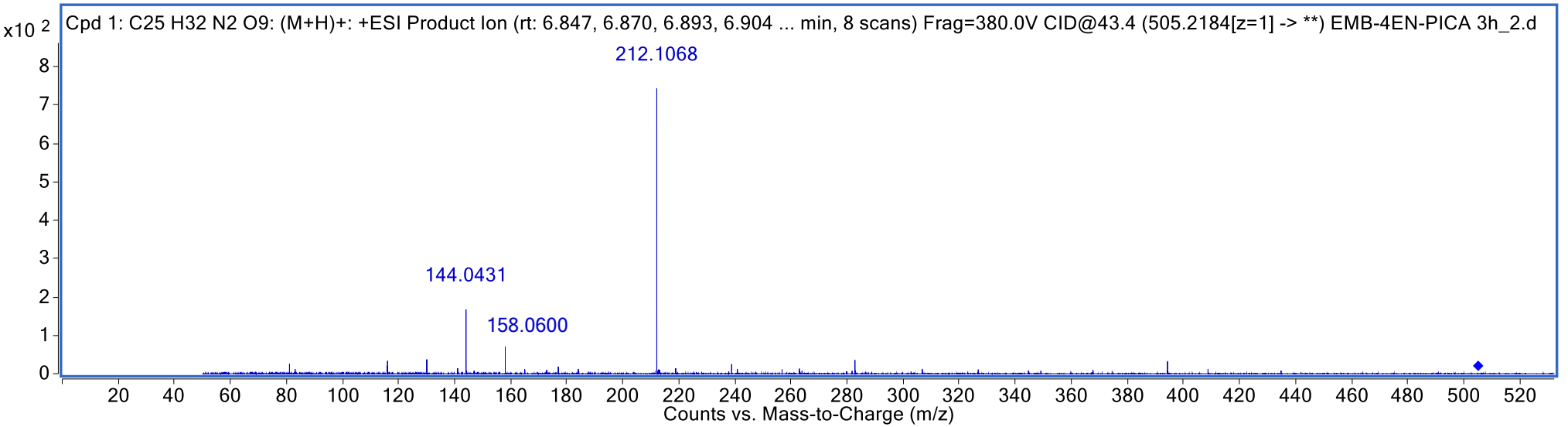


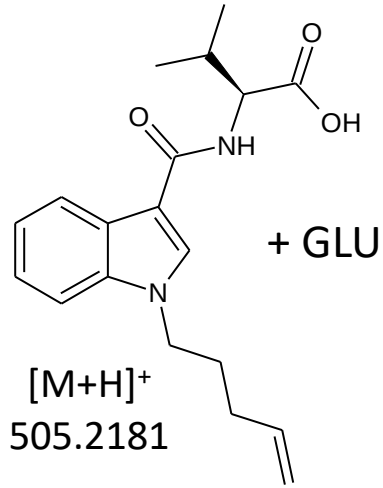




C3, Ester hydrolysis + glucuronidation, RT 6.86 min, *m/z* 505.2179



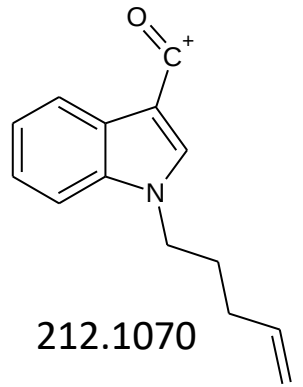




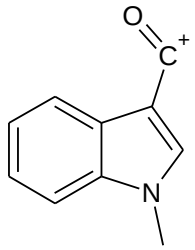
+ GLUC

$[M+H]^+$

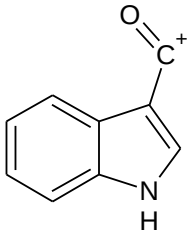
505.2181



212.1070

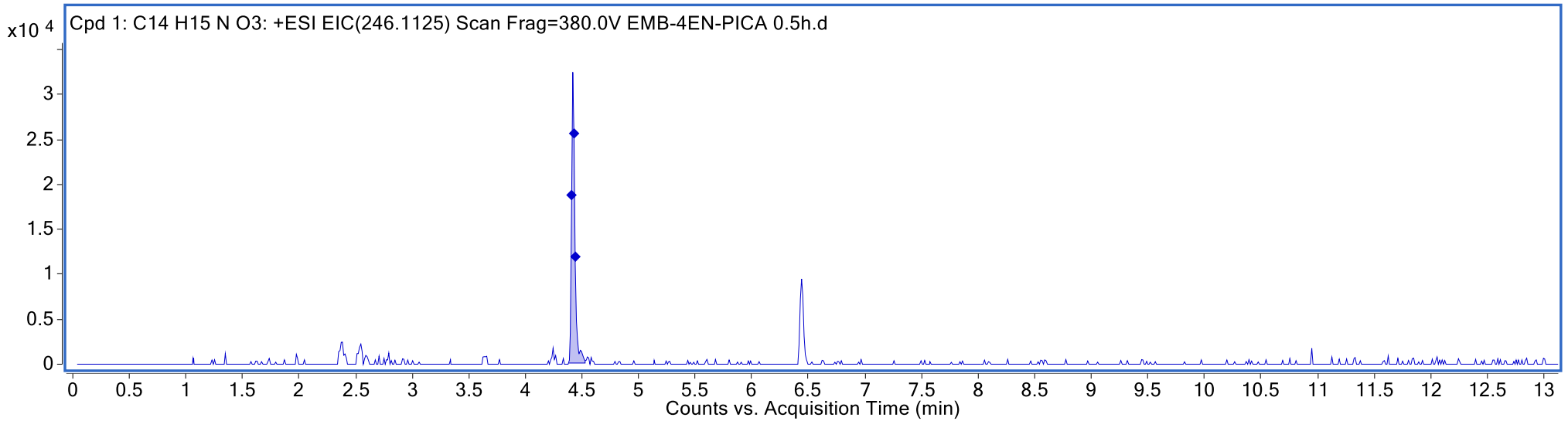


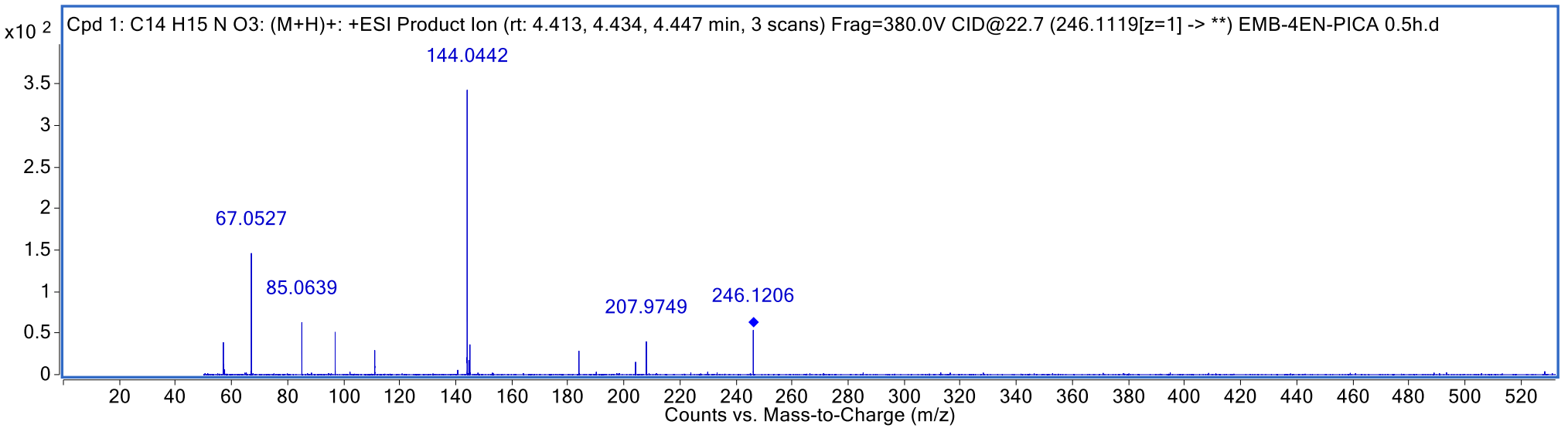
158.0600

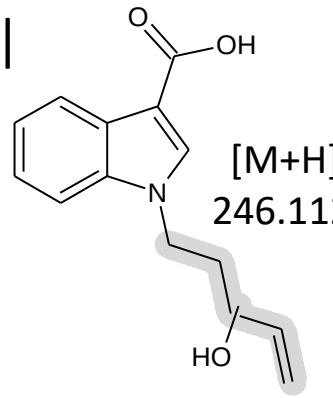


144.0444

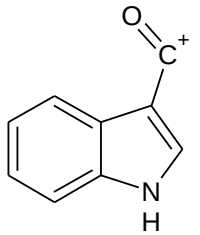
C4, Secondary amide hydrolysis + mono-hydroxylation (pentenyl tail), RT 4.42 min, *m/z* 246.1121



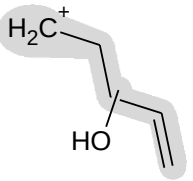




$[M+H]^+$
246.1125

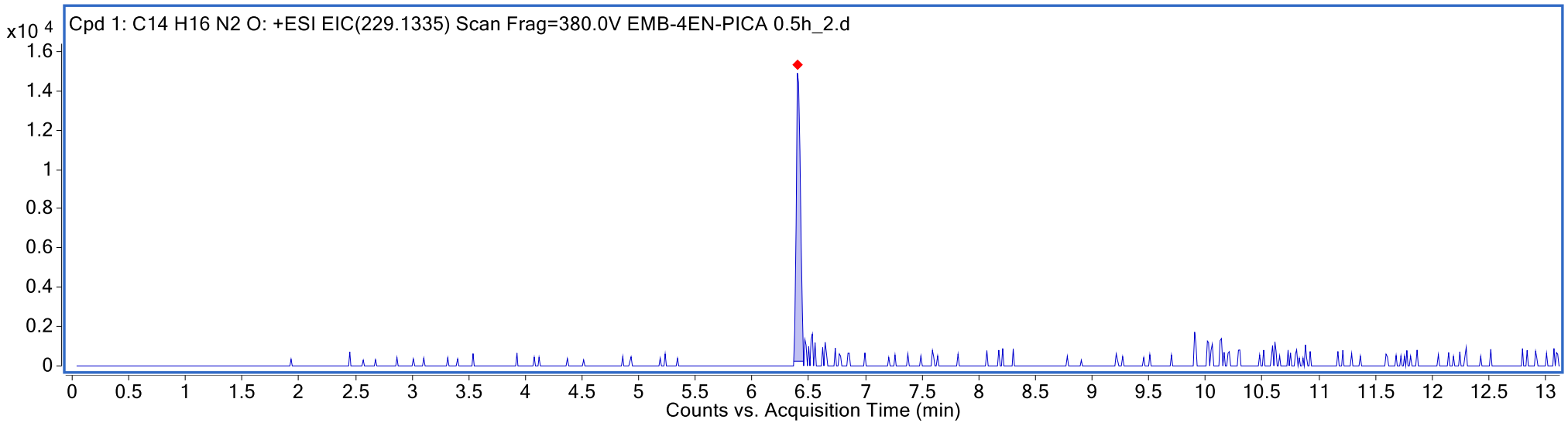


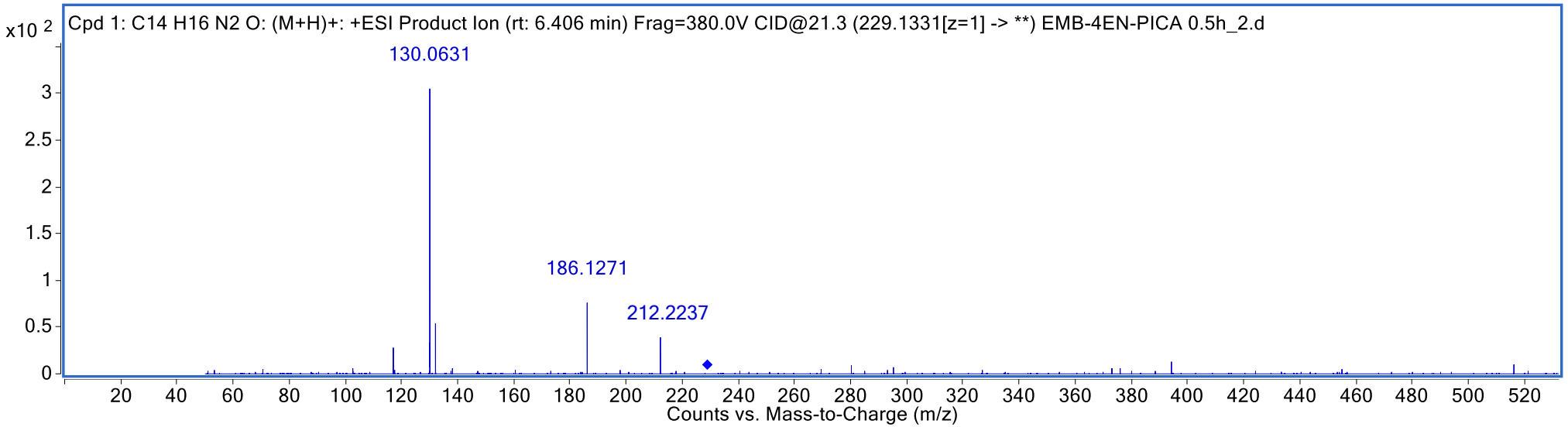
144.0444

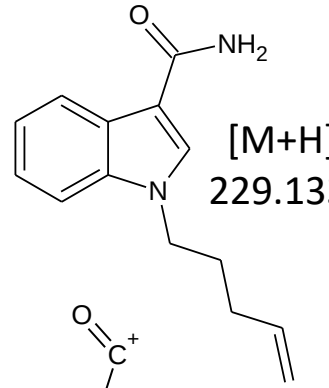


85.0648 $\xrightarrow{-H_2O}$ 67.0542

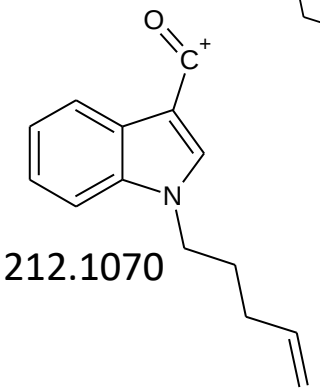
C5, Head group–linker cleavage, RT 6.41 min, *m/z* 229.1330



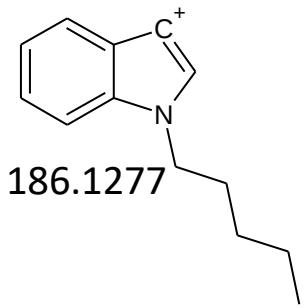




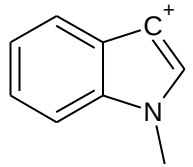
[M+H]+
229.1335



212.1070

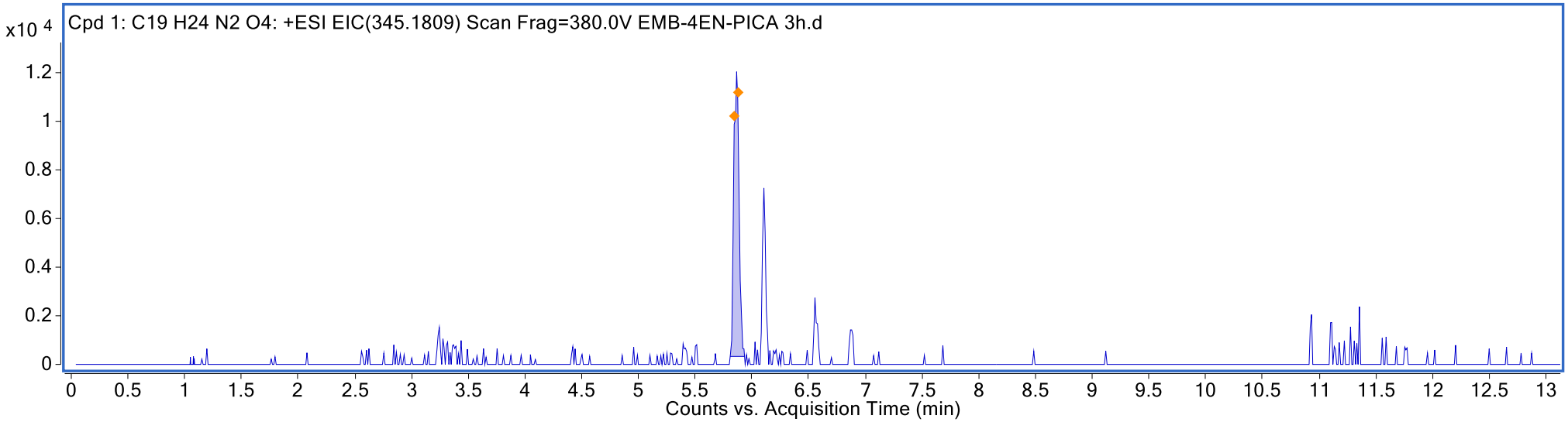


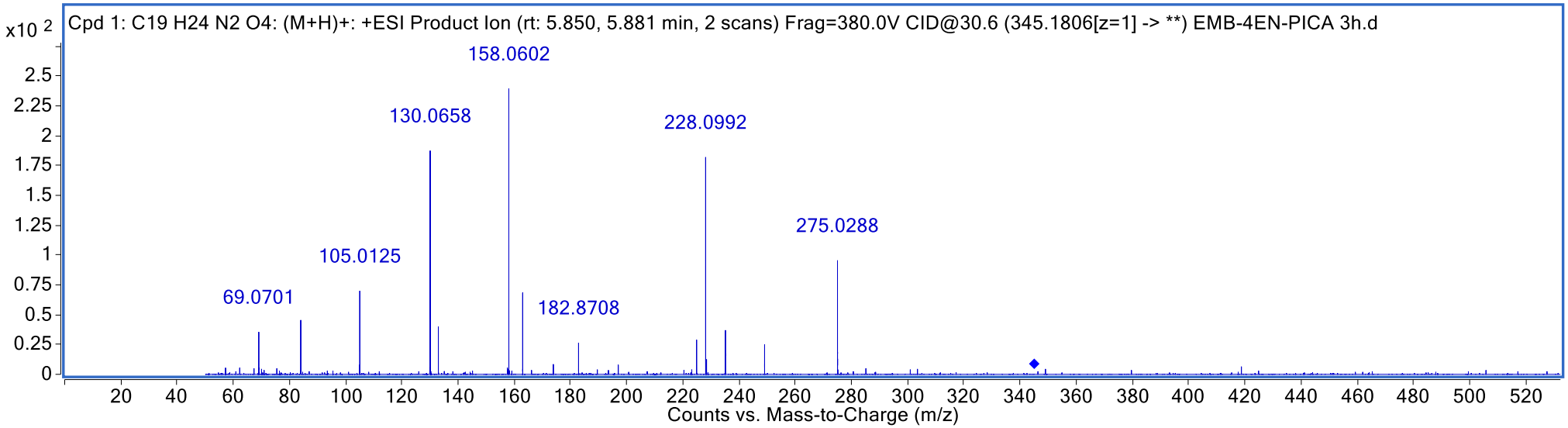
186.1277

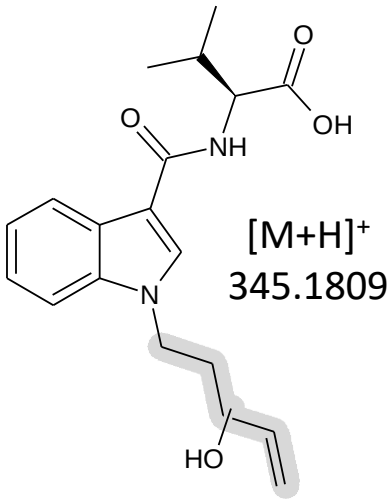


130.0651

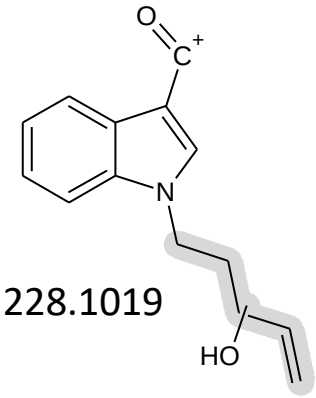
C6, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 5.87 min, *m/z* 345.1806



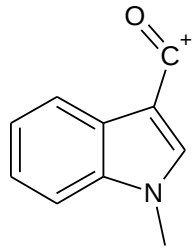




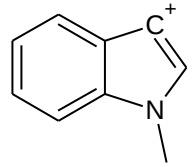
[M+H]+
345.1809



228.1019

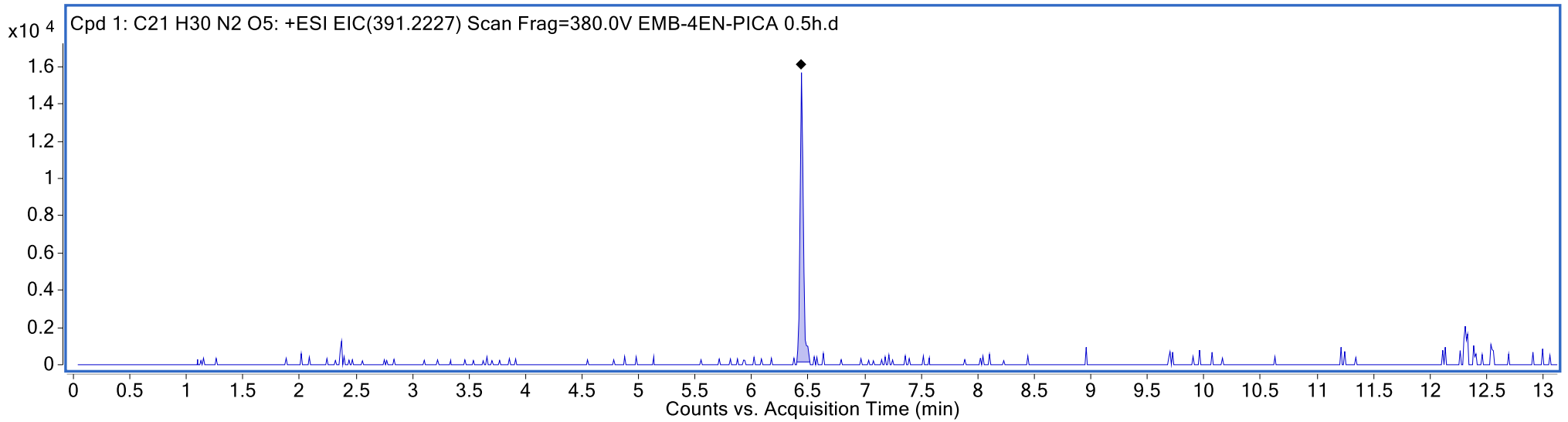


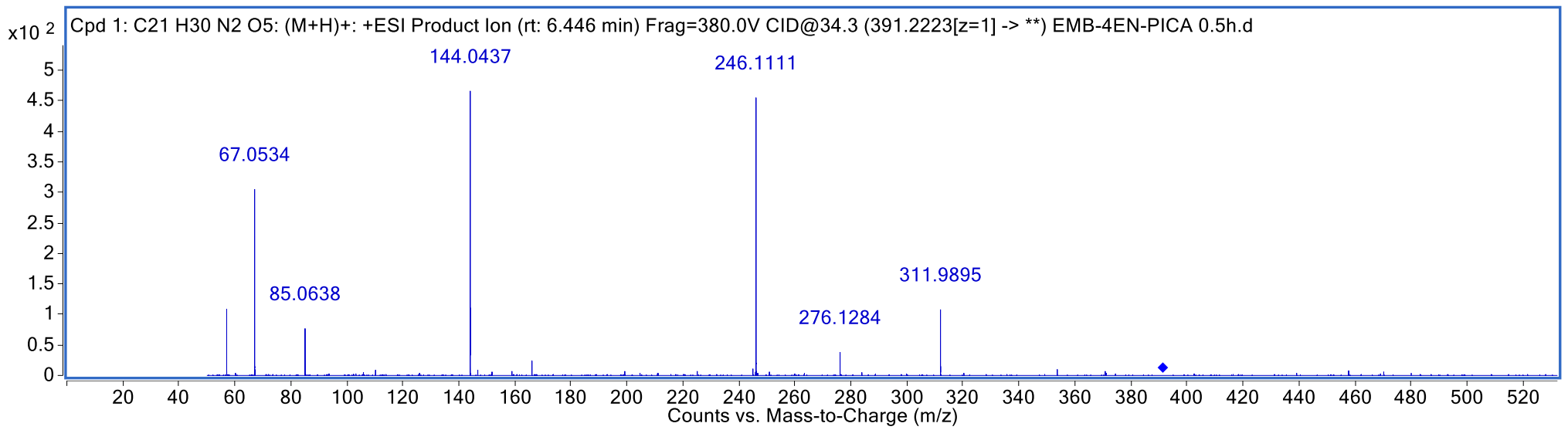
158.0600

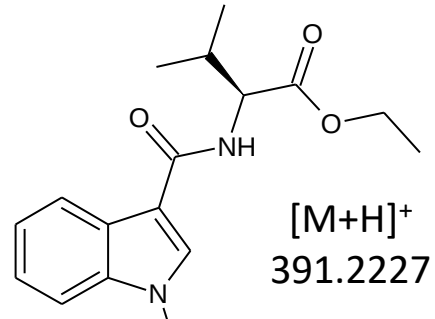


130.0651

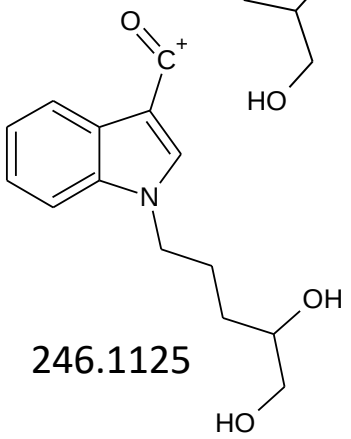
C7, Dihydrodiol formation, RT 6.44 min, *m/z* 391.2225



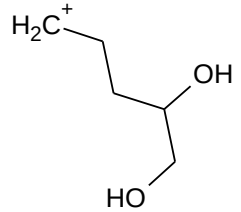




$[M+H]^+$
391.2227

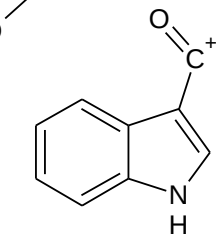


246.1125



103.0754
(not detected)

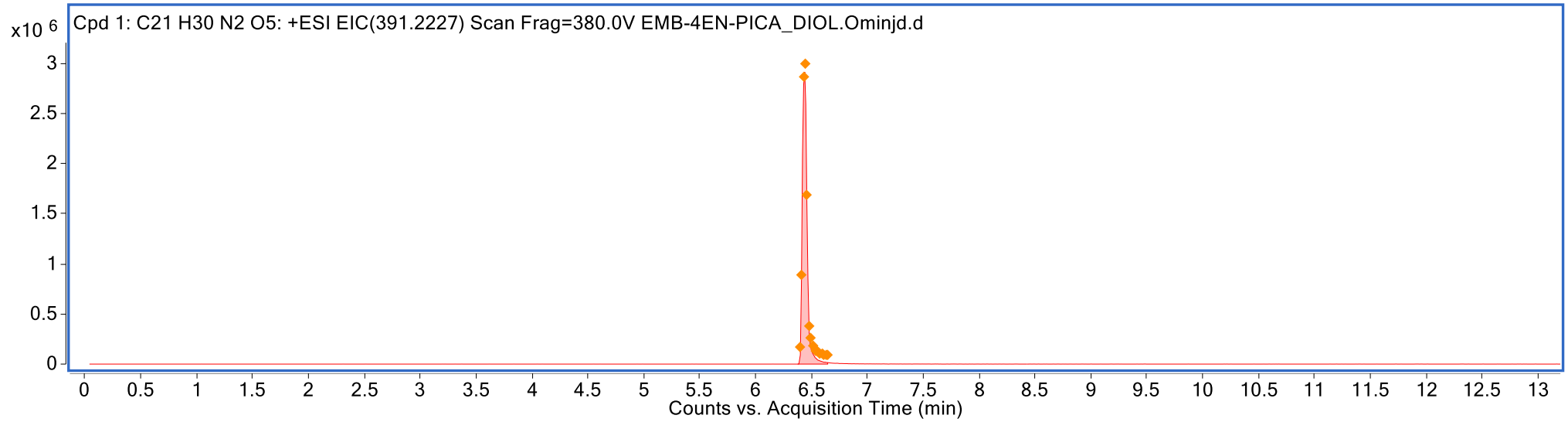
$-H_2O$

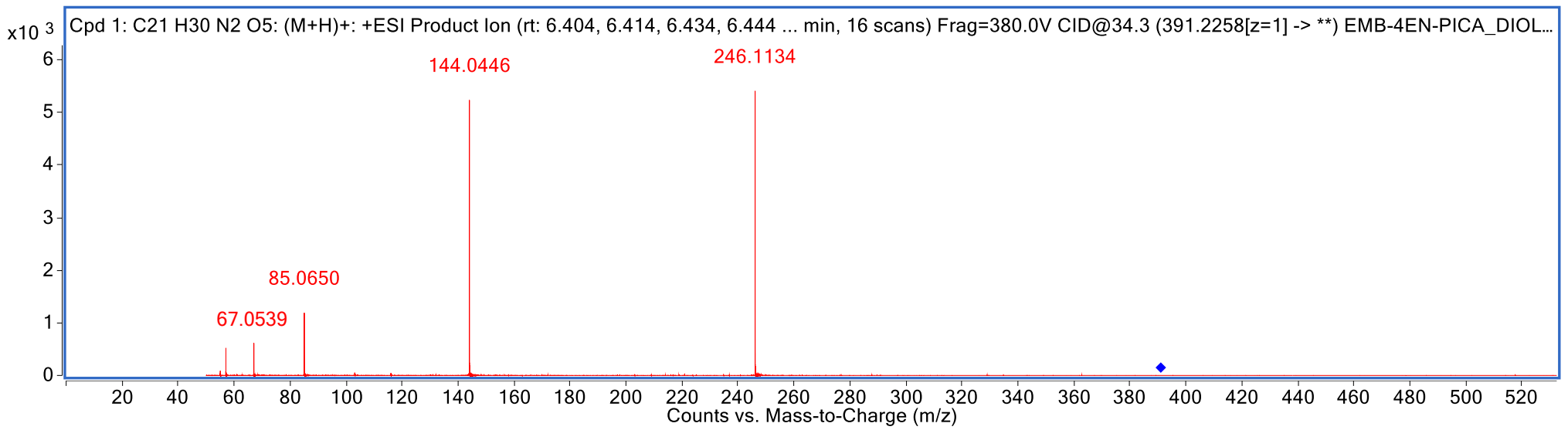


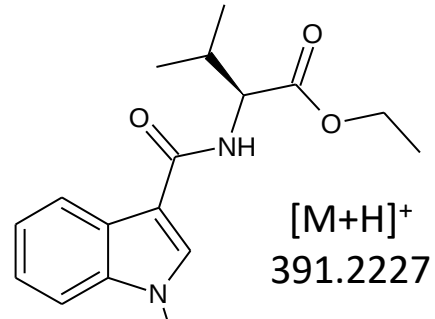
144.0444

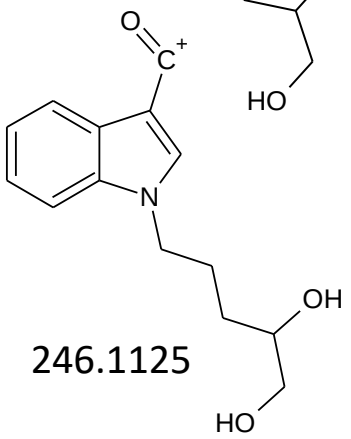
85.0648 $\xrightarrow{-H_2O}$ 67.0542

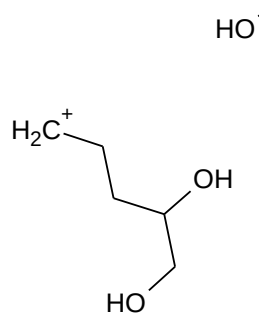
Dihydrodiol reference standard, RT 6.44 min, *m/z* 391.2258

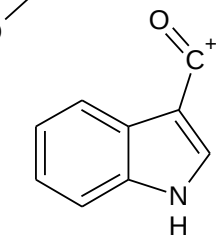




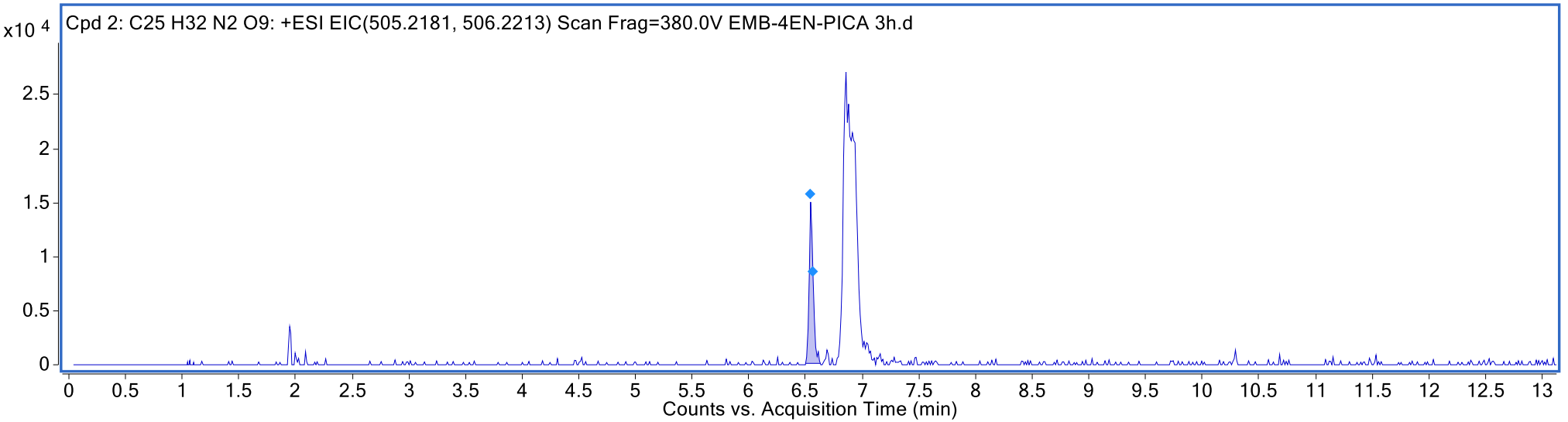


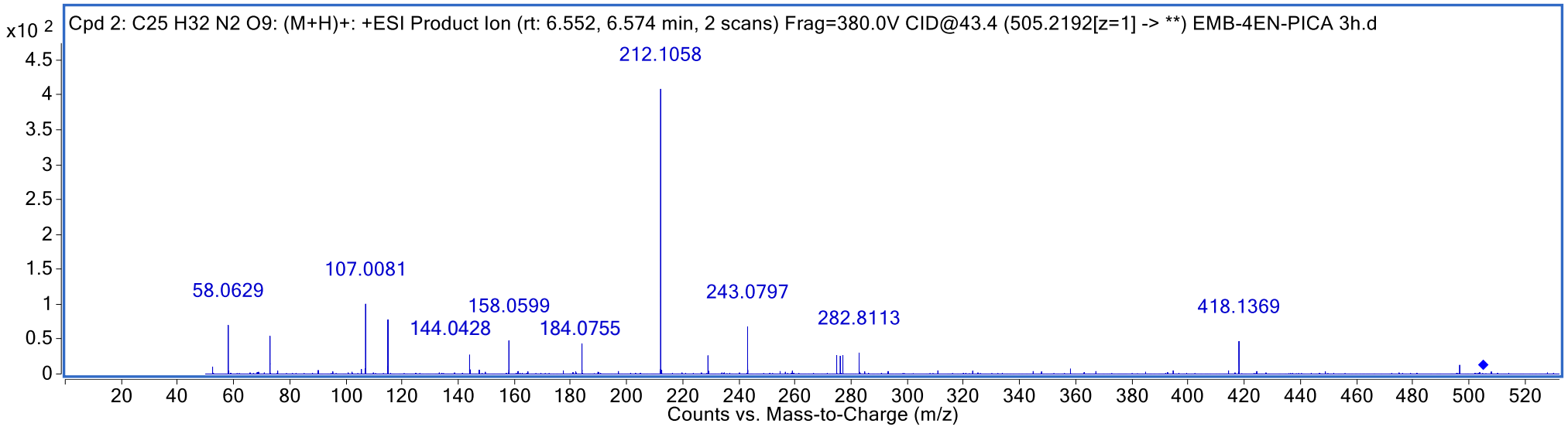


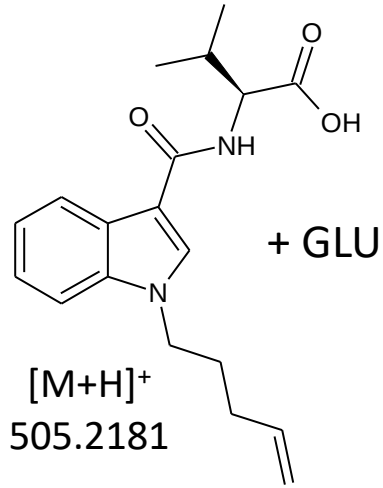




C8, Ester hydrolysis + glucuronidation, RT 6.55 min, *m/z* 505.2193

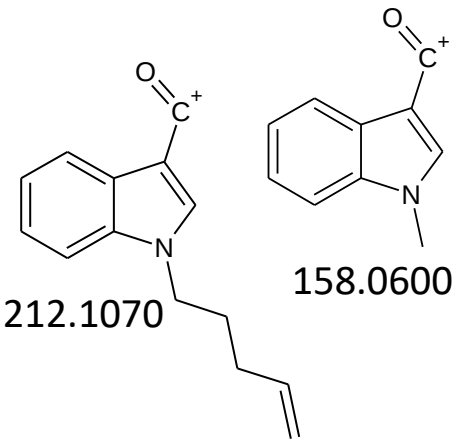






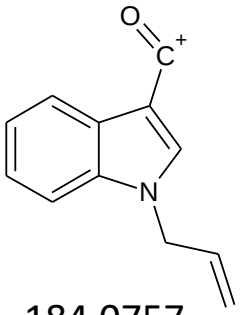
+ GLUC

$[M+H]^+$
505.2181

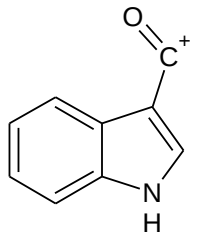


212.1070

158.0600



184.0757

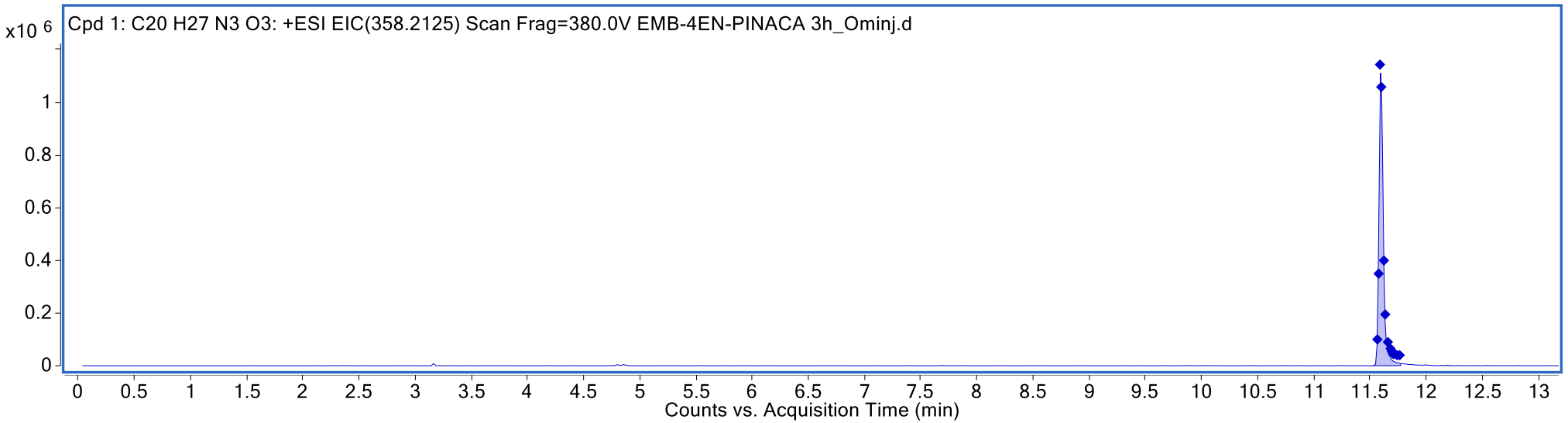


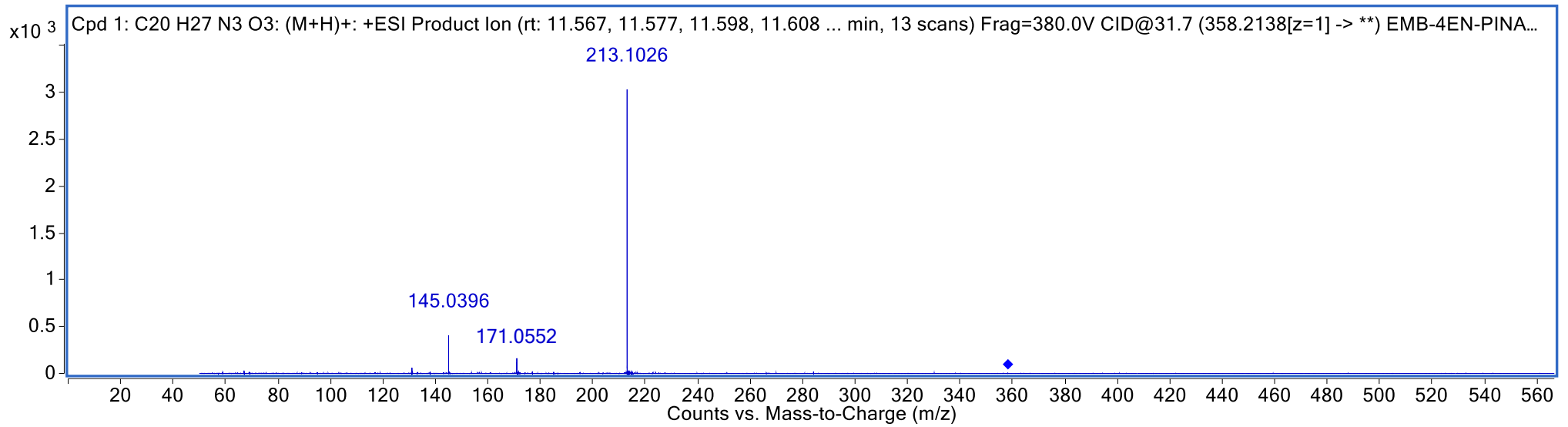
144.0444

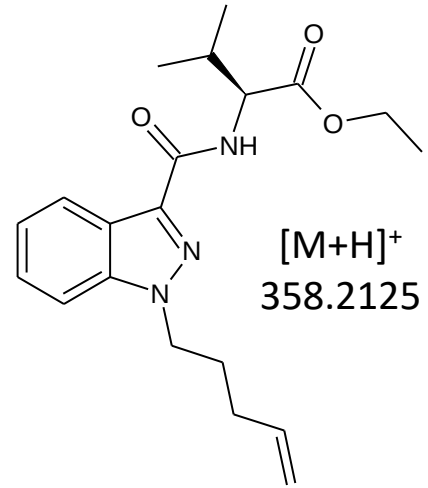
EMB-4en-PINACA

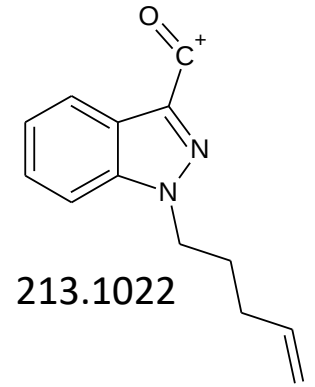
Metabolism

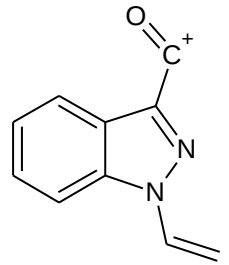
EMB-4en-PINACA, RT 11.61 min, *m/z* 358.2146

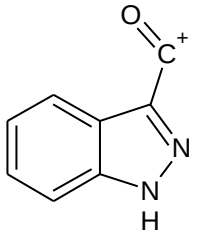




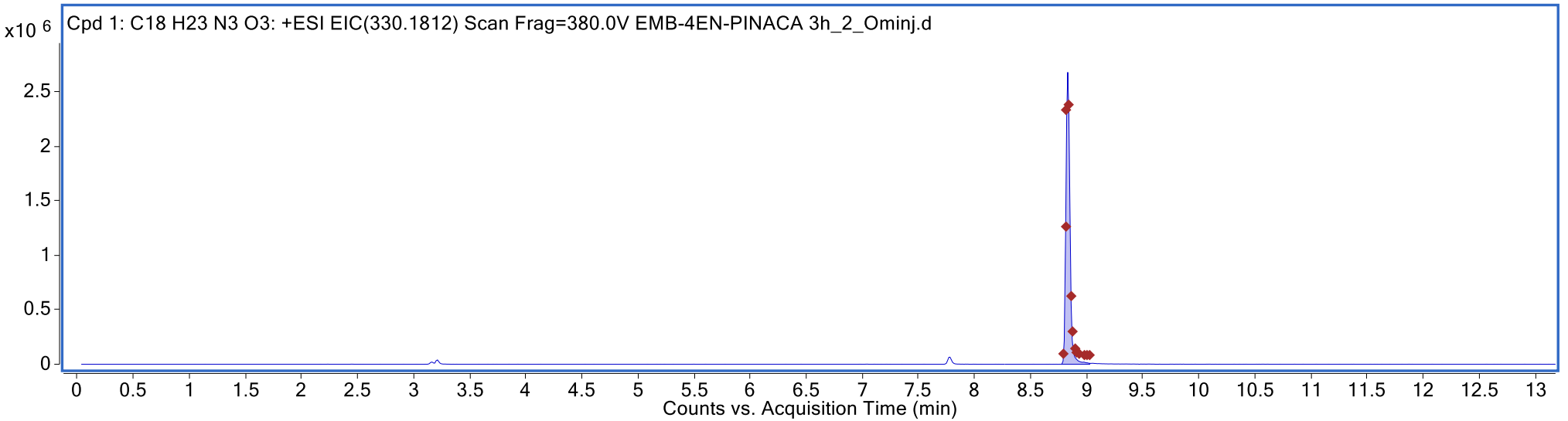


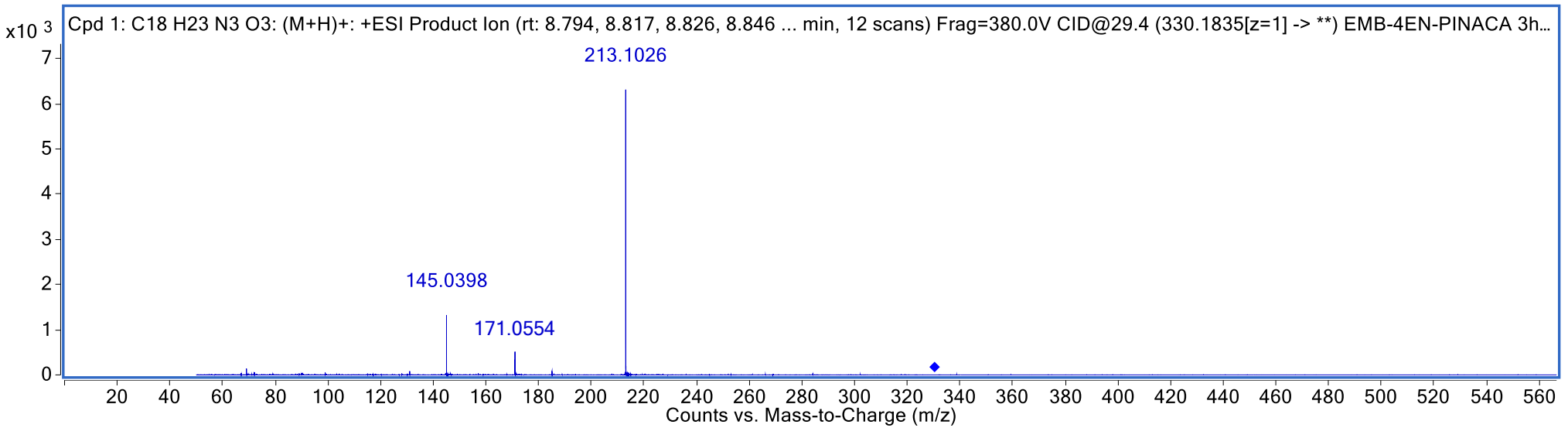


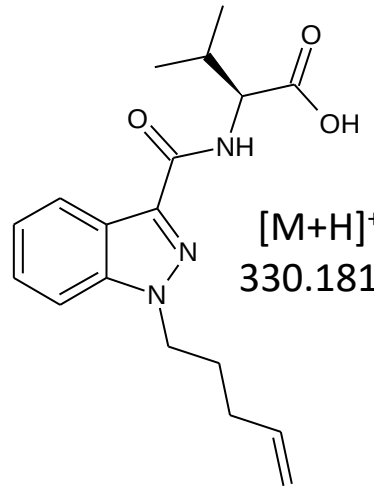




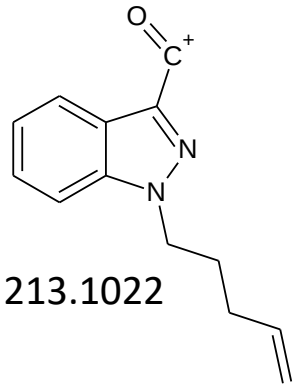
D1, Ester hydrolysis, RT 8.84 min, *m/z* 330.1834



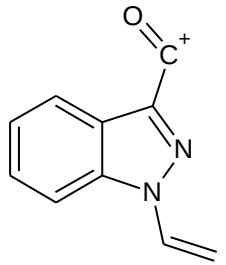




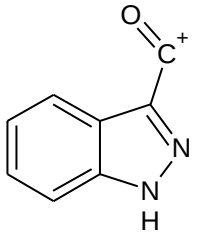
[M+H]+
330.1812



213.1022

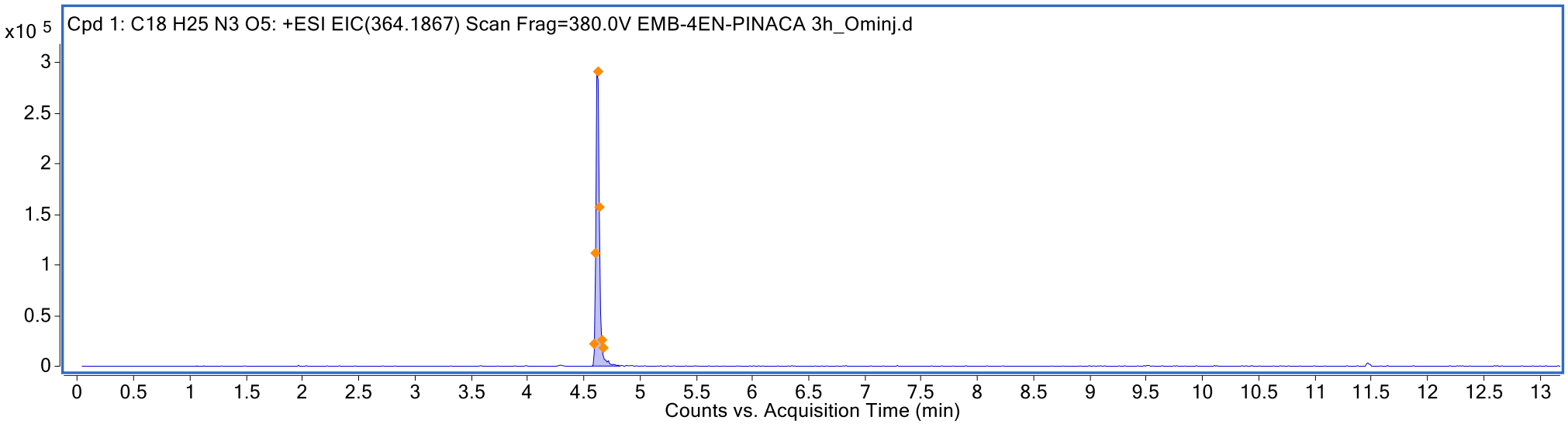


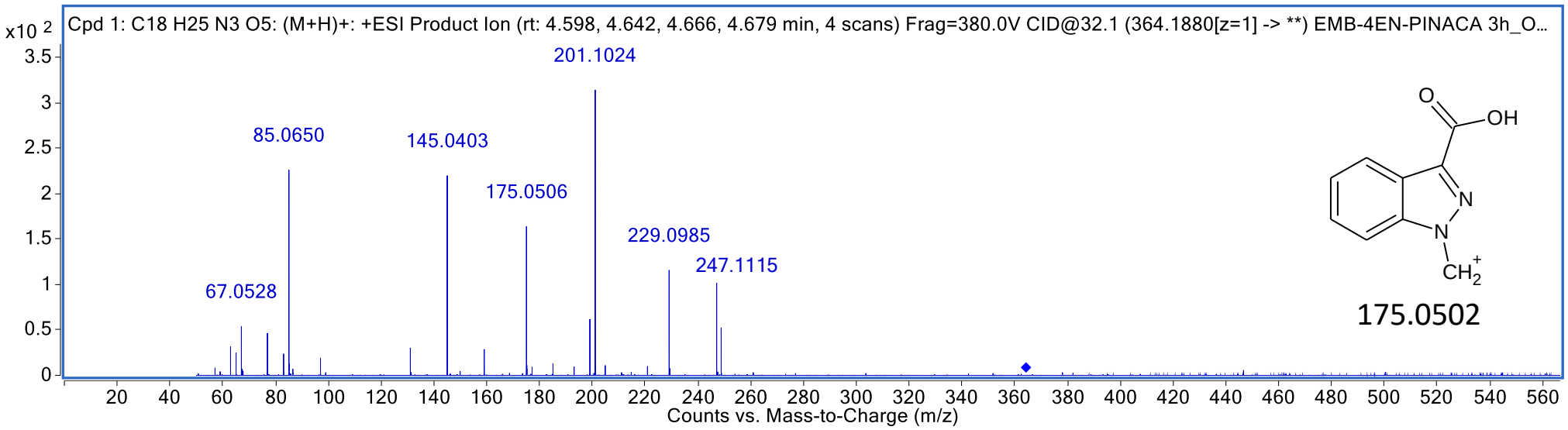
171.0553

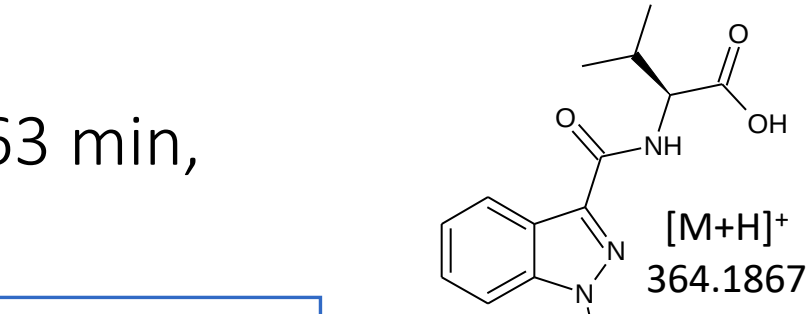


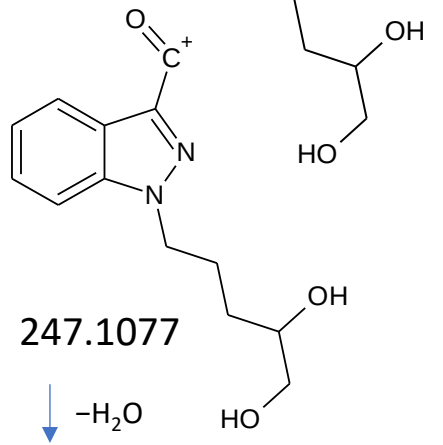
145.0396

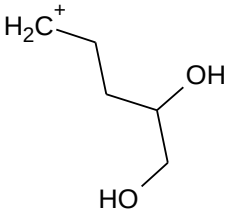
D2, Ester hydrolysis + dihydrodiol formation, RT 4.63 min, *m/z* 364.1875

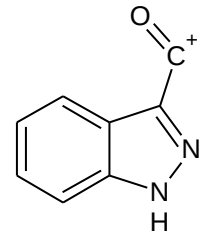




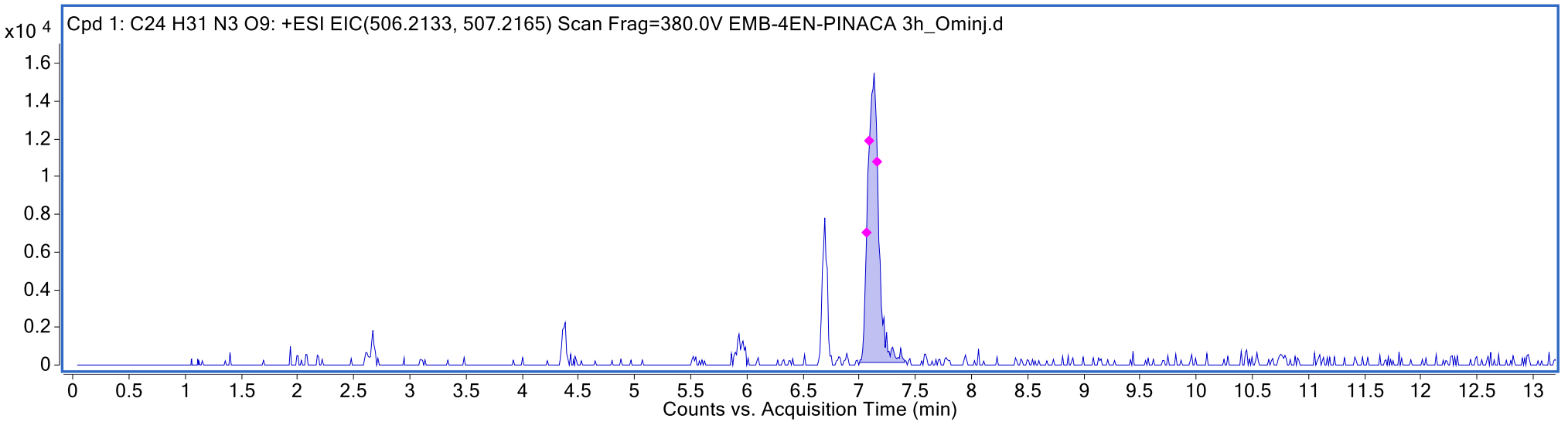


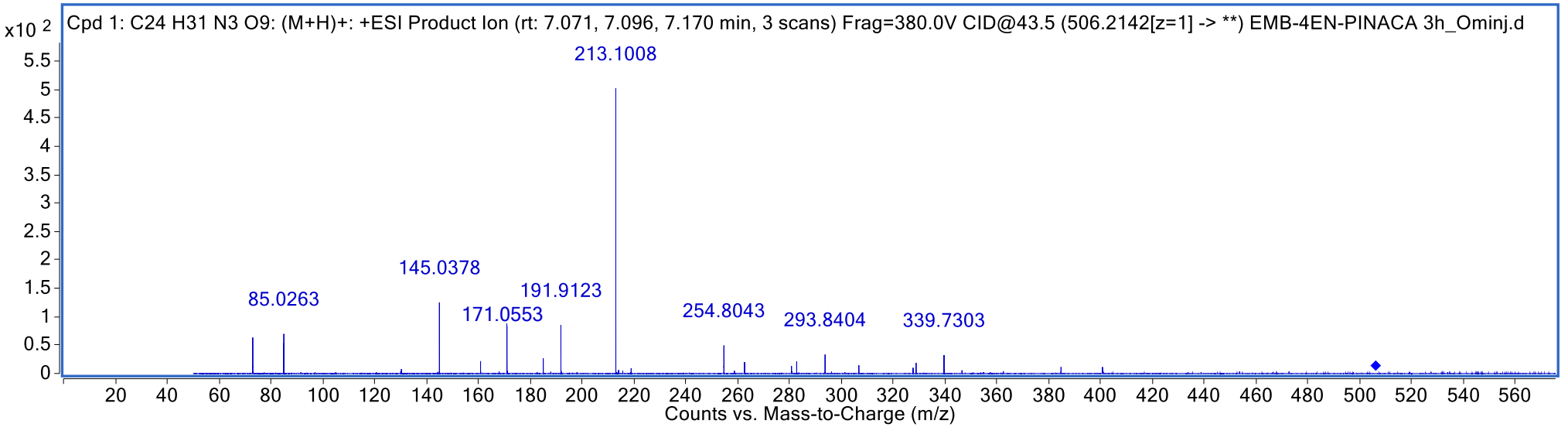


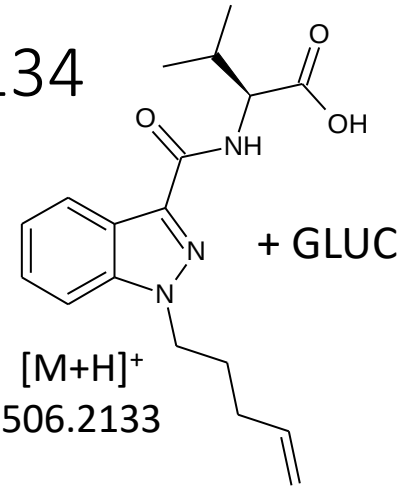




D3, Ester hydrolysis + glucuronidation, RT 7.14 min, *m/z* 506.2134

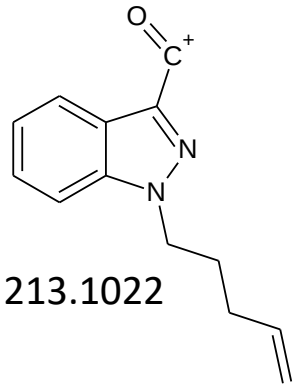




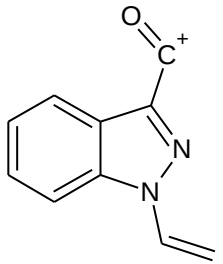


+ GLUC

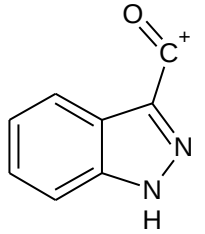
$[M+H]^+$
506.2133



213.1022

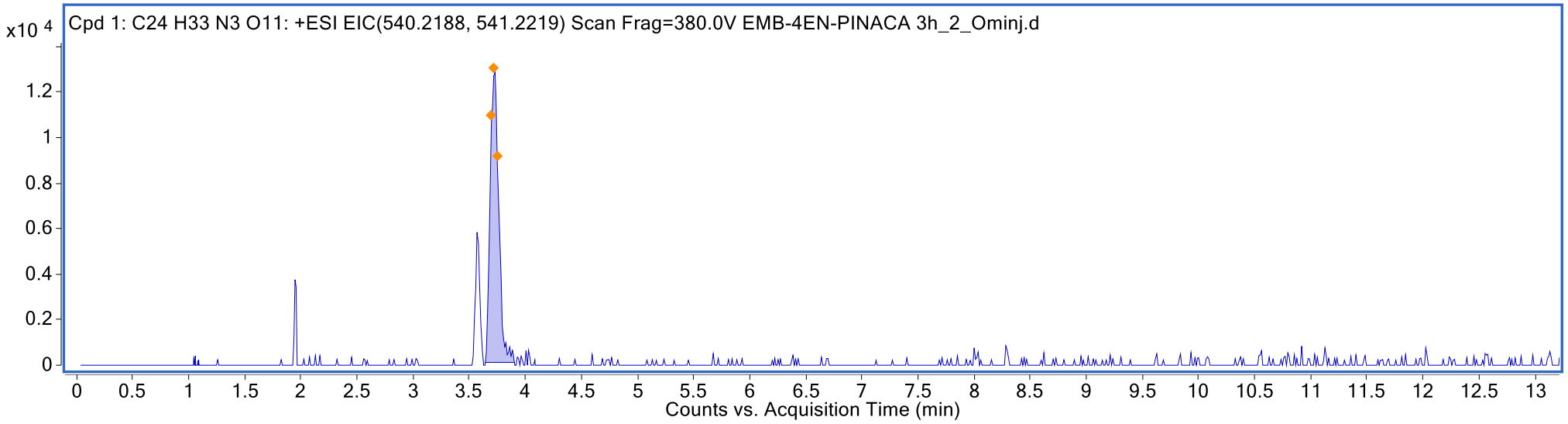


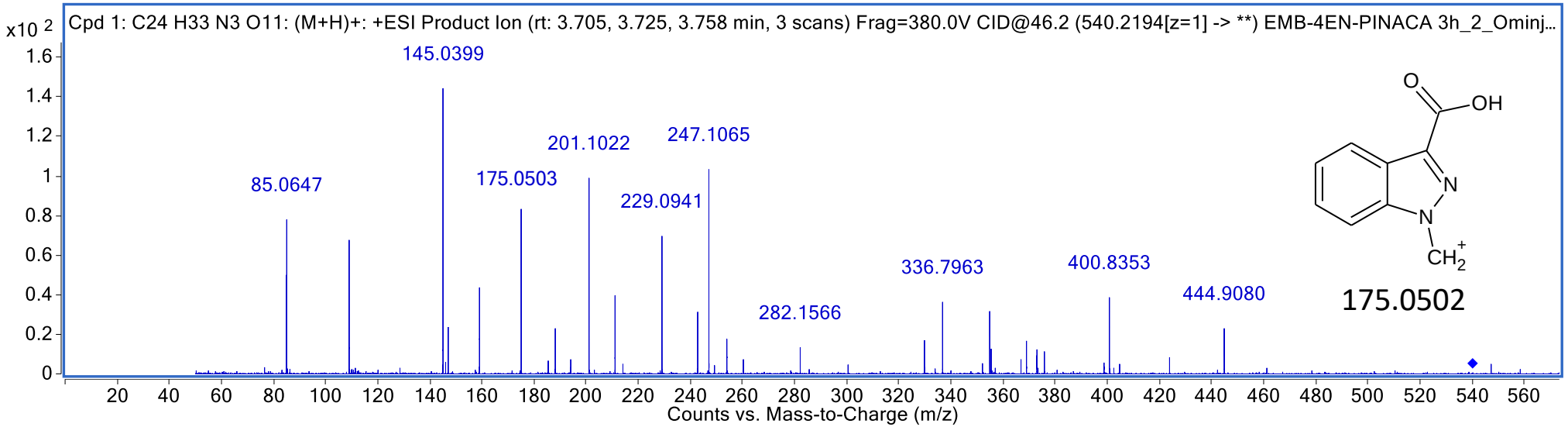
171.0553

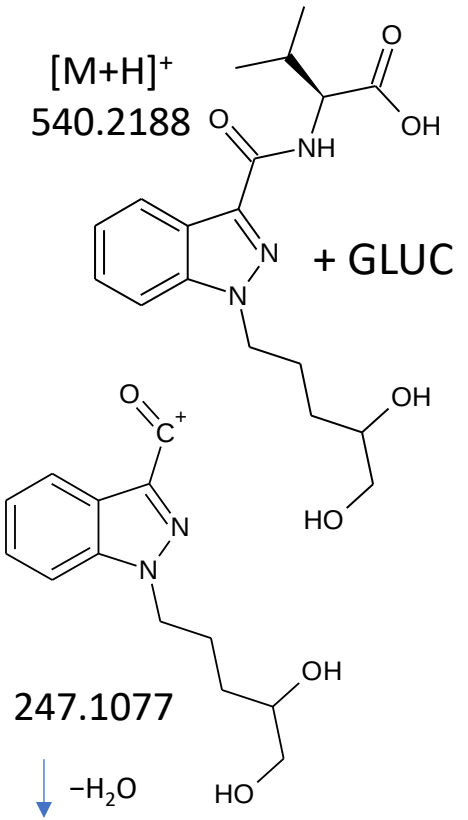


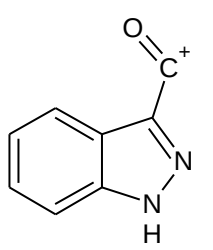
145.0396

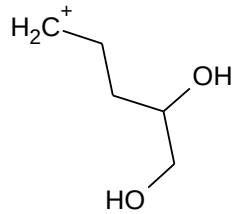
D4, Ester hydrolysis + dihydrodiol formation + glucuronidation, RT 3.73 min, *m/z* 540.2190



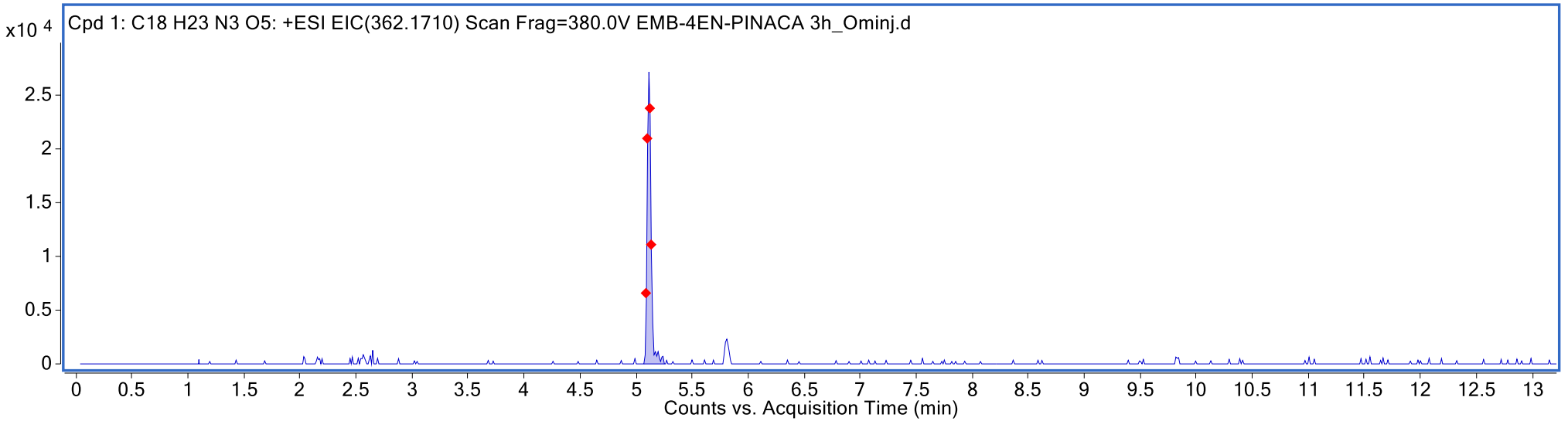


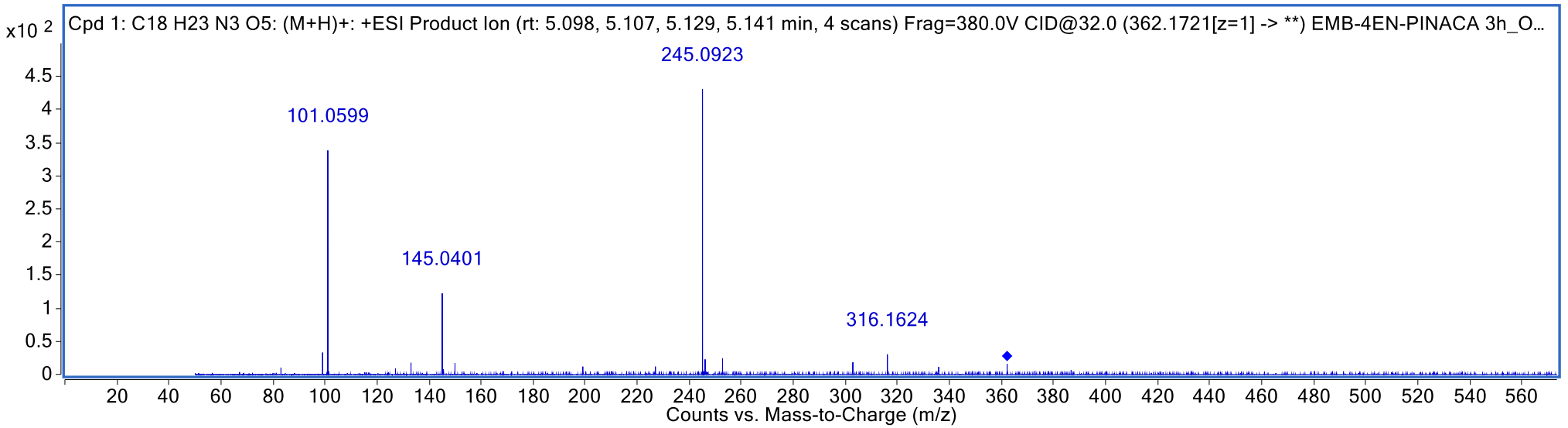


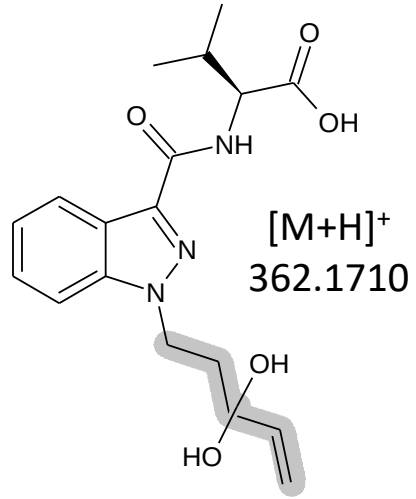


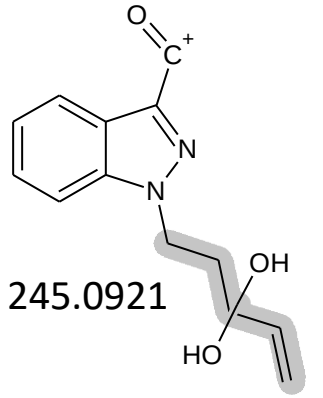


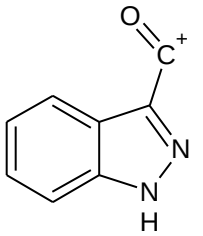
D5, Ester hydrolysis + di-hydroxylation (pentenyl tail), RT 5.12 min, *m/z* 362.1714

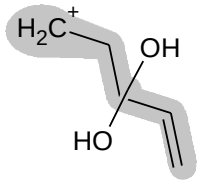




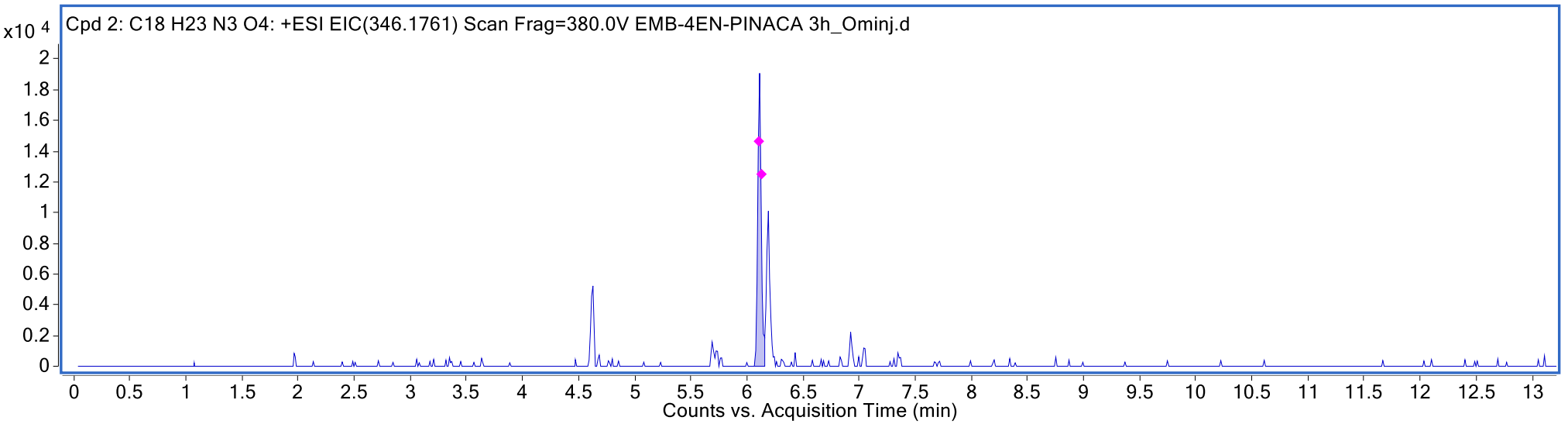


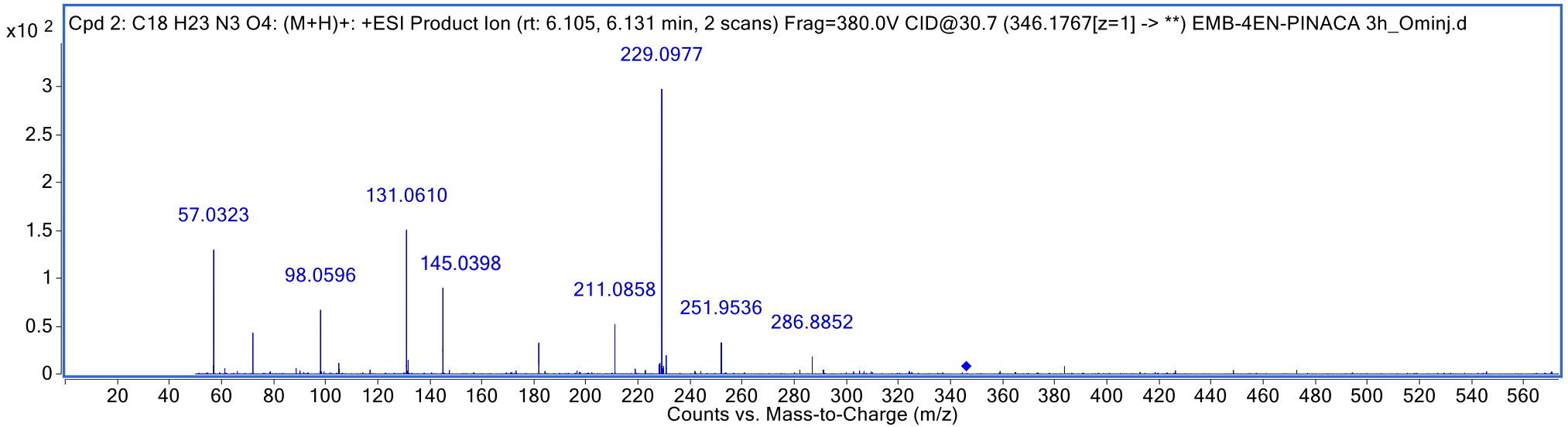


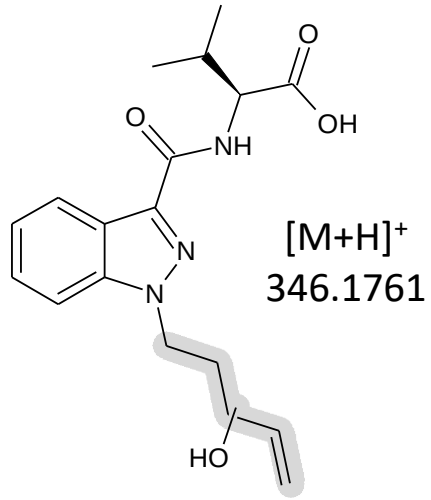




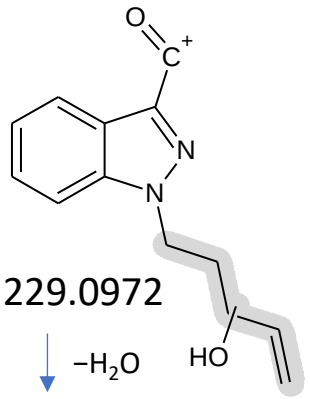
D6, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 6.11 min, *m/z* 346.1764







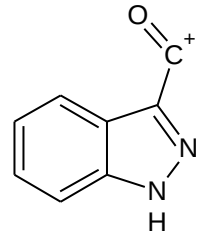
$[M+H]^+$
346.1761



229.0972

$-H_2O$

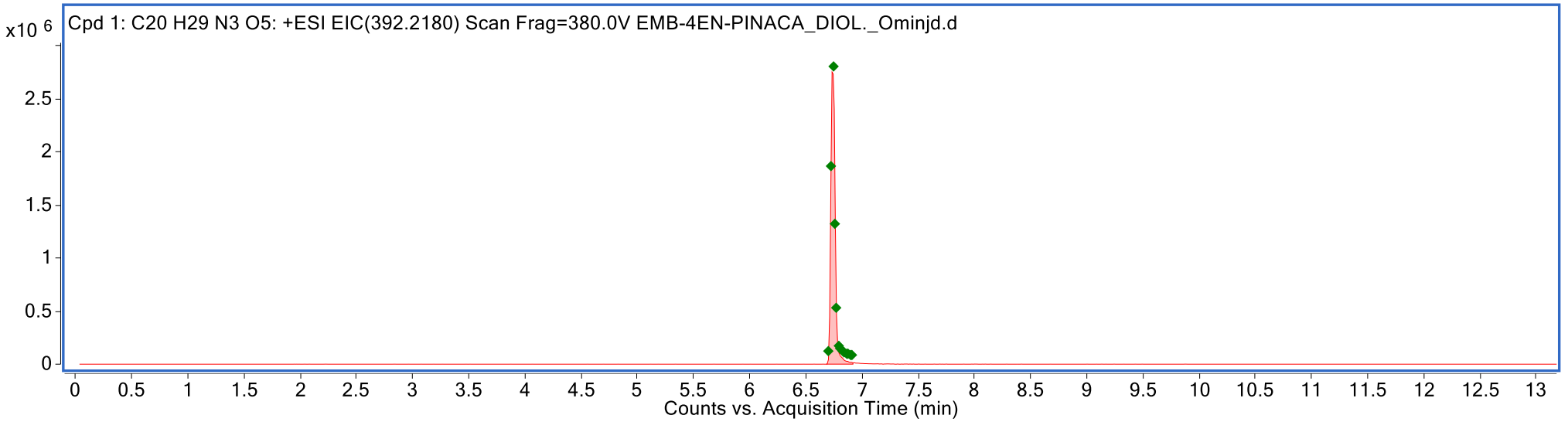
211.0866

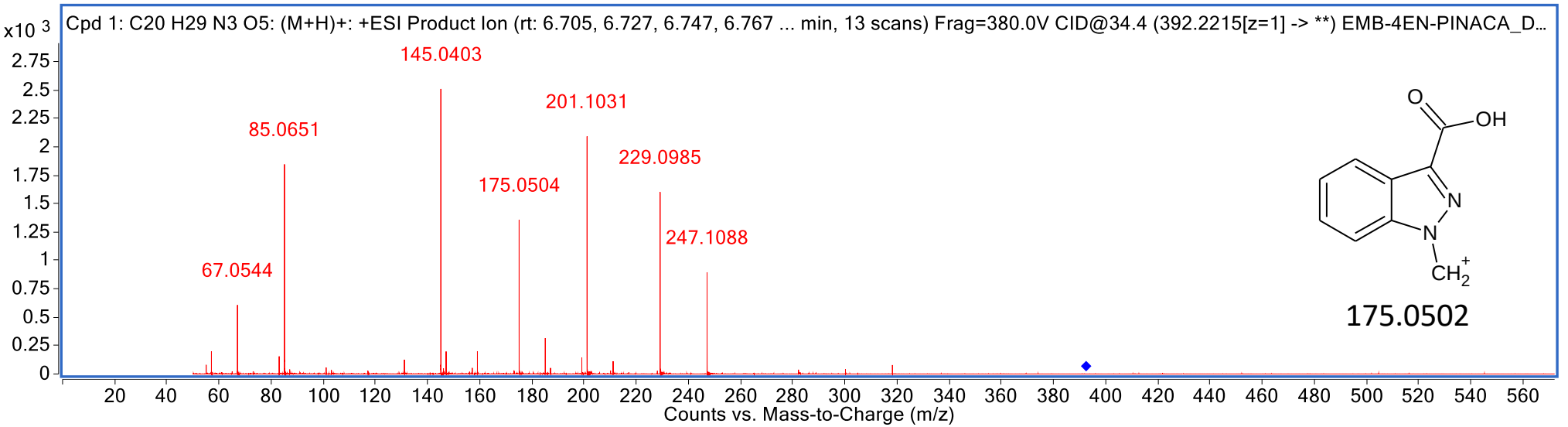


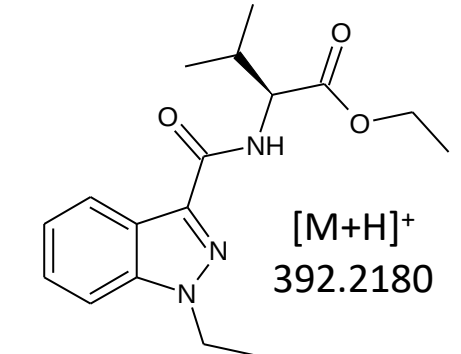
145.0396

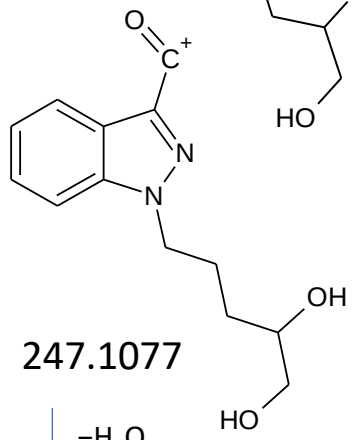
Dihydrodiol reference standard, RT 6.73 min, *m/z* 392.2215

(not detected in HHep incubations)



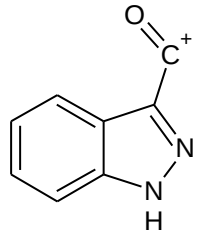


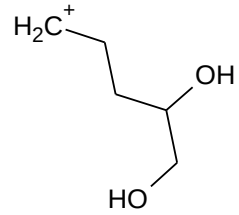




−H_2O

229.0972 → 201.1022 (−CO)

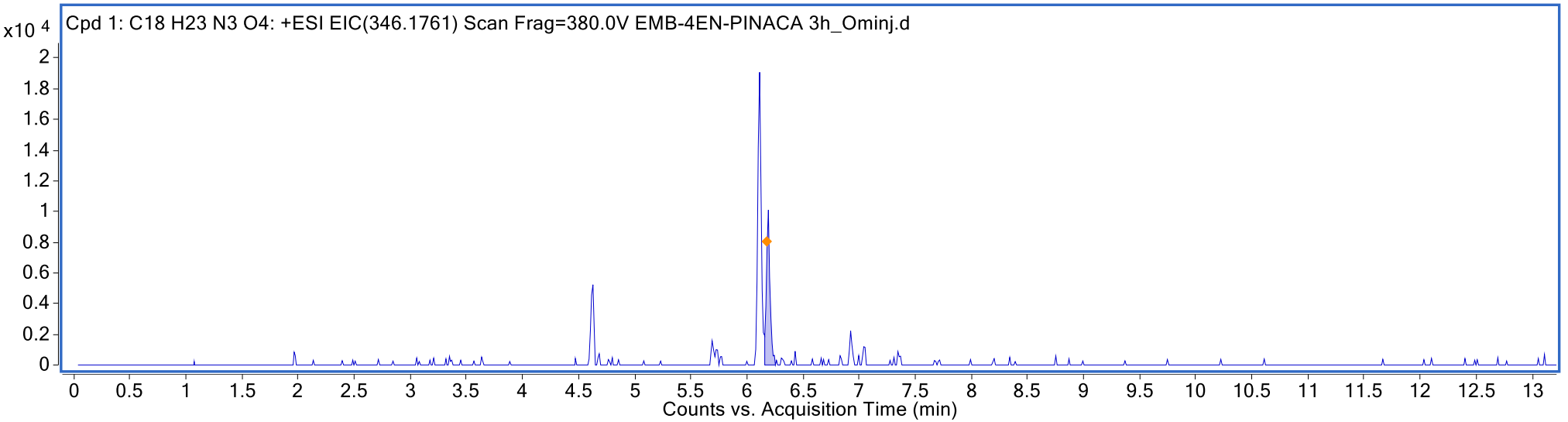


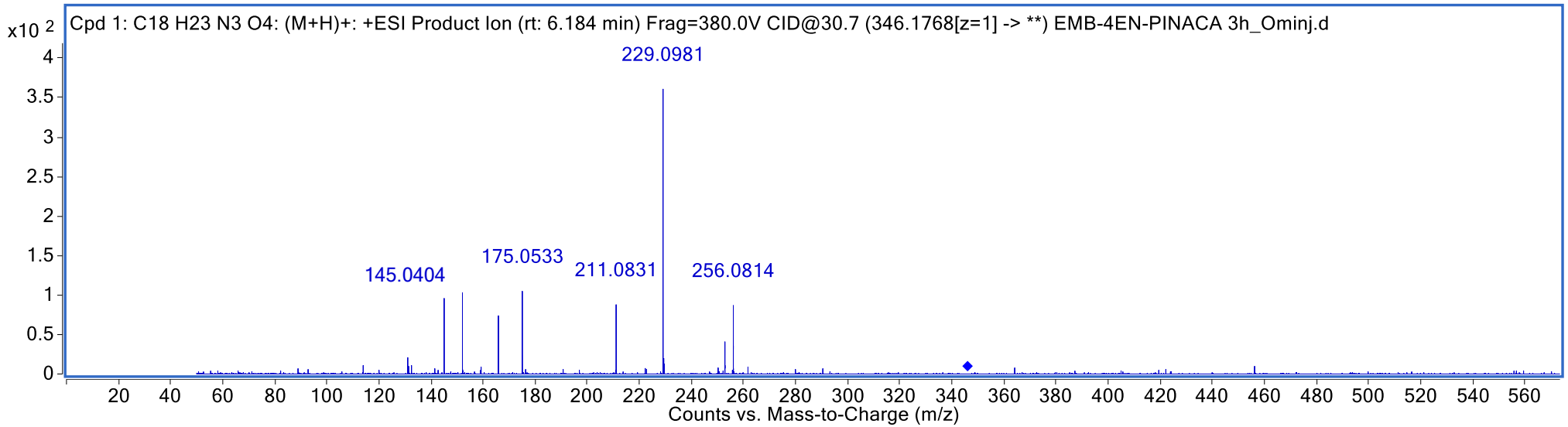


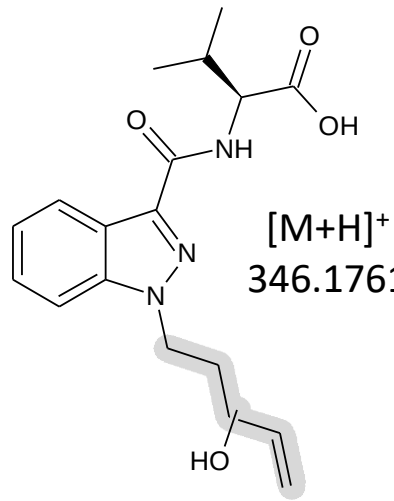
−H_2O

85.0648

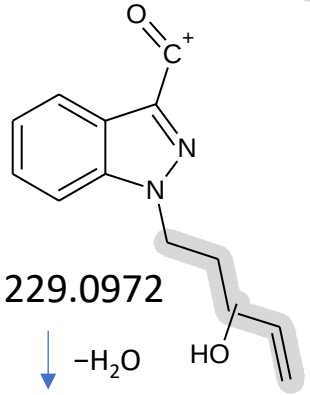
D7, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 6.19 min, *m/z* 346.1770







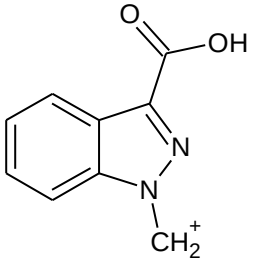
[M+H]+
346.1761



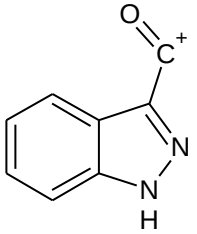
229.0972

$-H_2O$

211.0866



175.0502

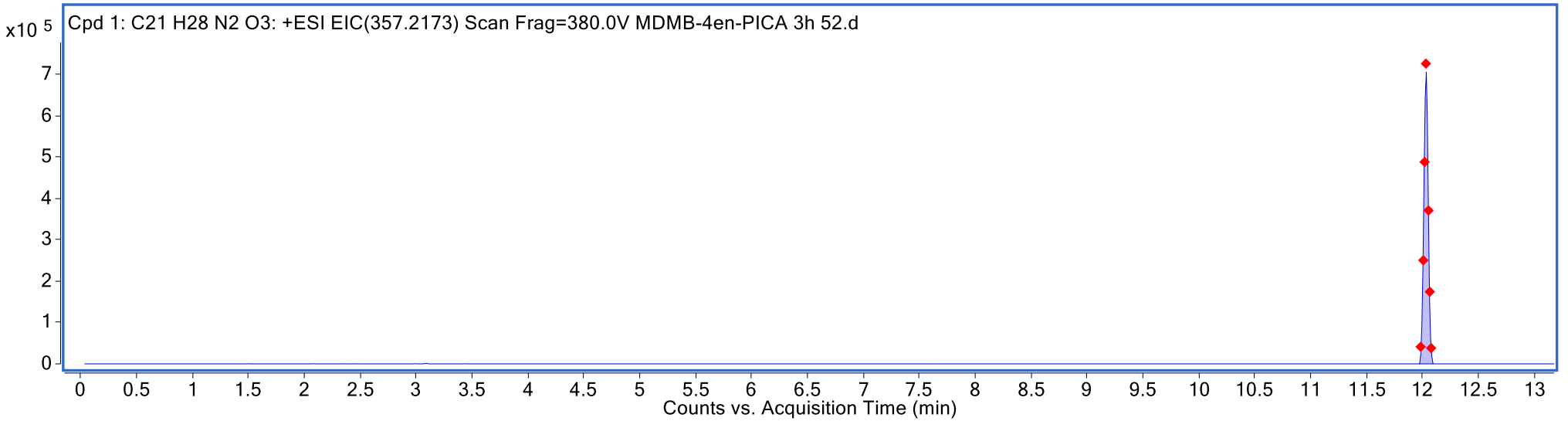


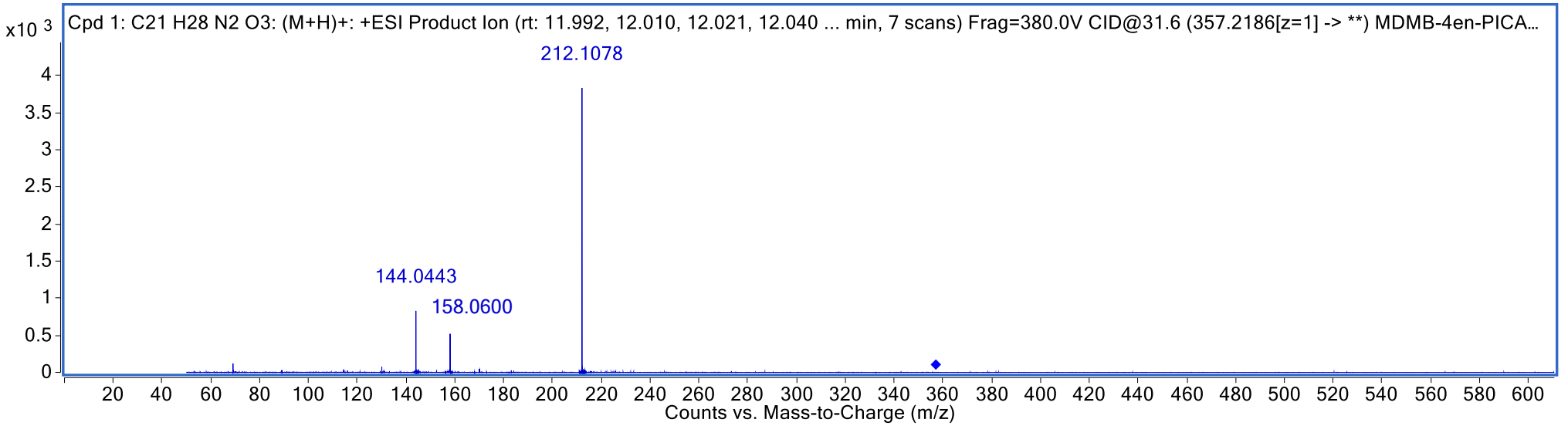
145.0396

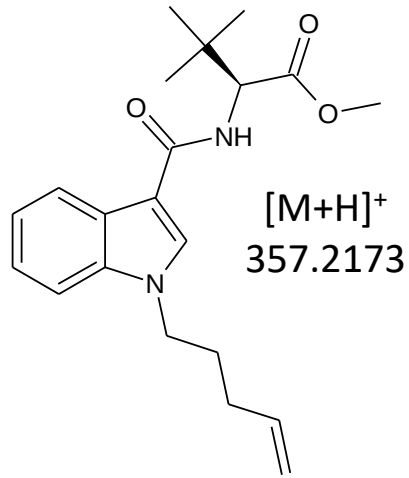
MDMB-4en-PICA

Metabolism

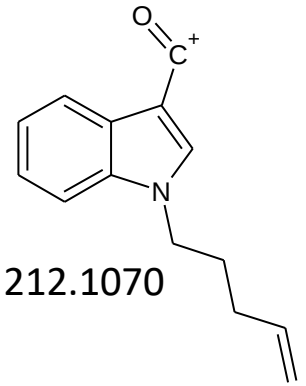
MDMB-4en-PICA, RT 12.04 min, *m/z* 357.2209



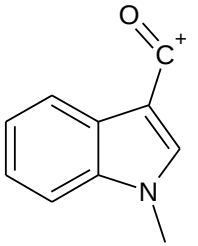




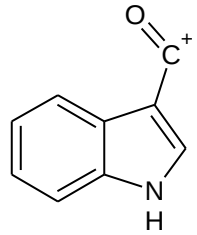
[M+H]+
357.2173



212.1070

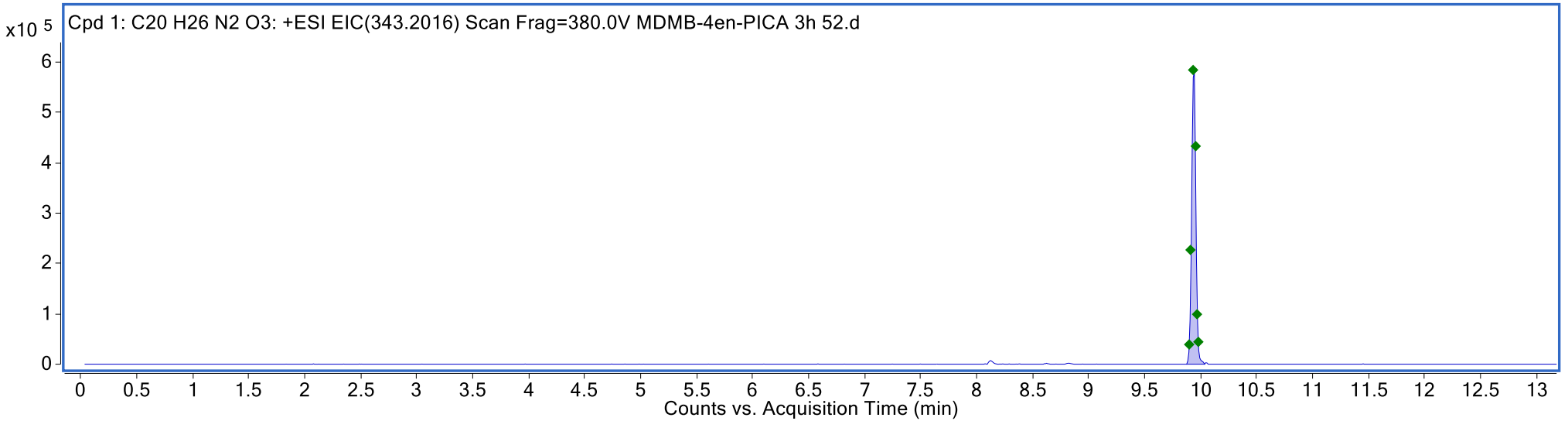


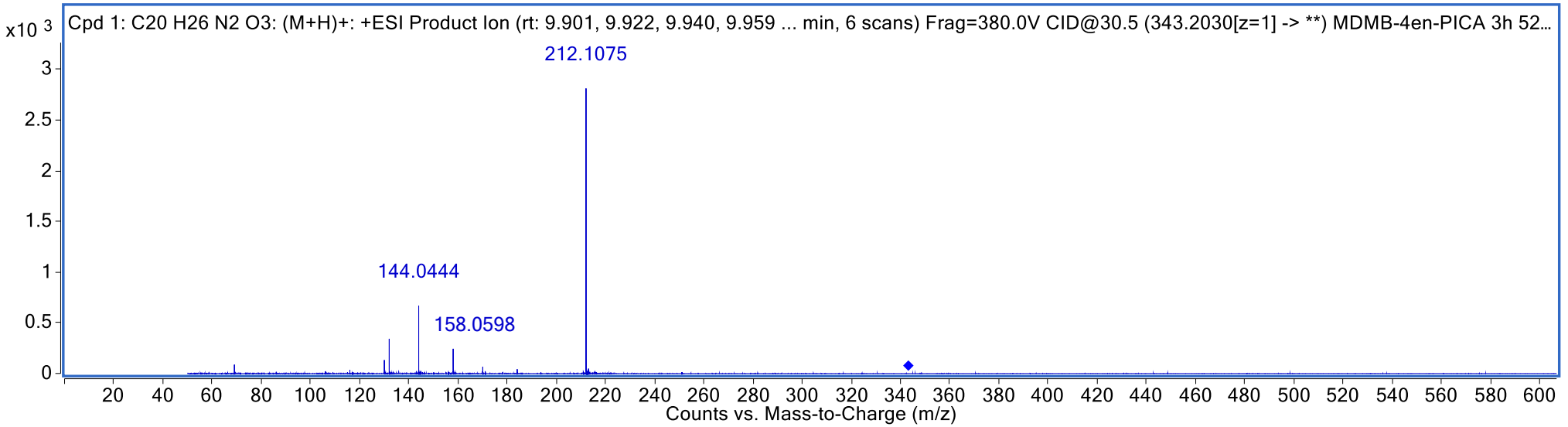
158.0600

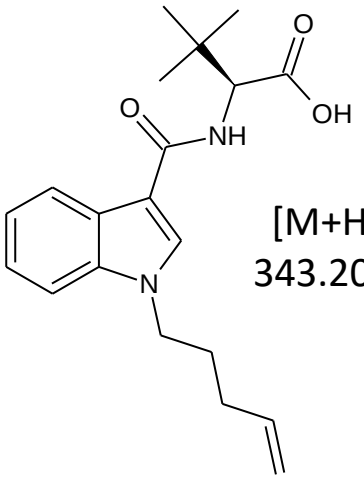


144.0444

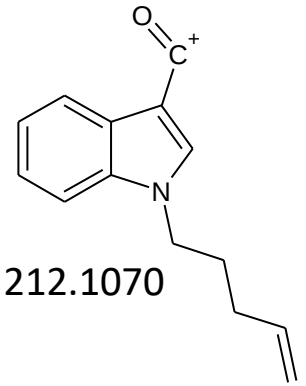
E1, Ester hydrolysis, RT 9.94 min, *m/z* 343.2026



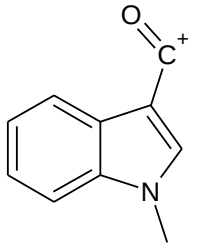




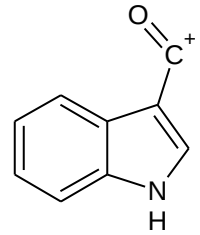
[M+H]+
343.2016



212.1070

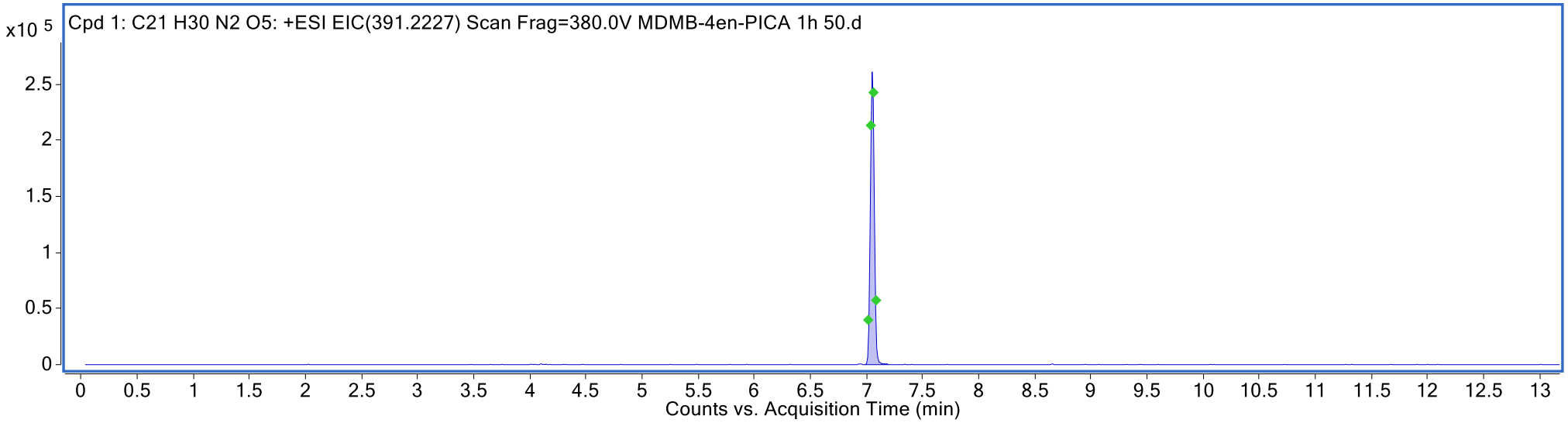


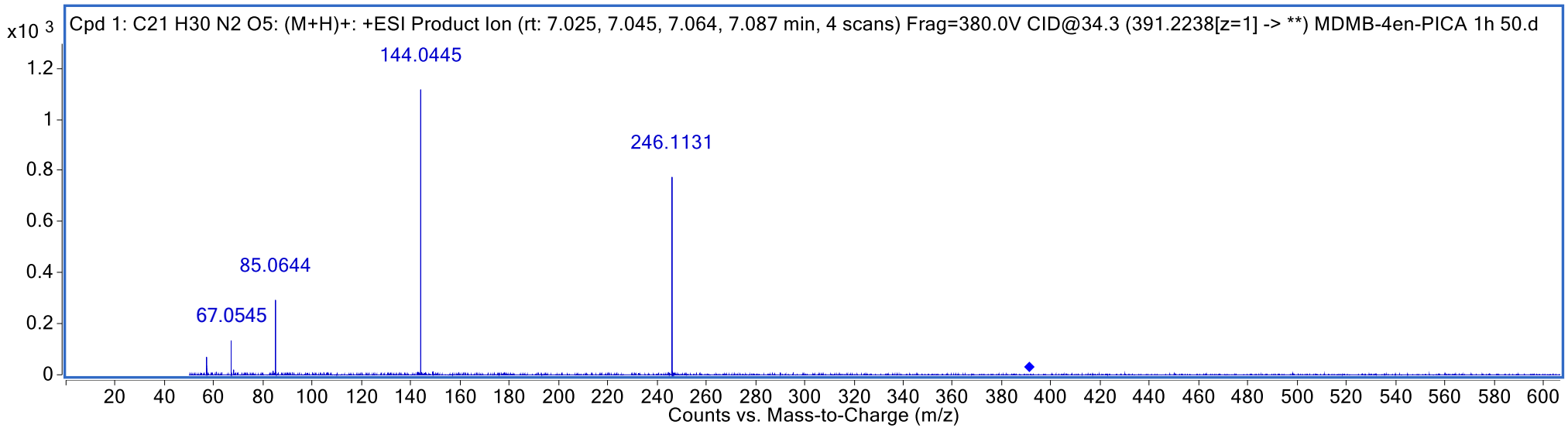
158.0600

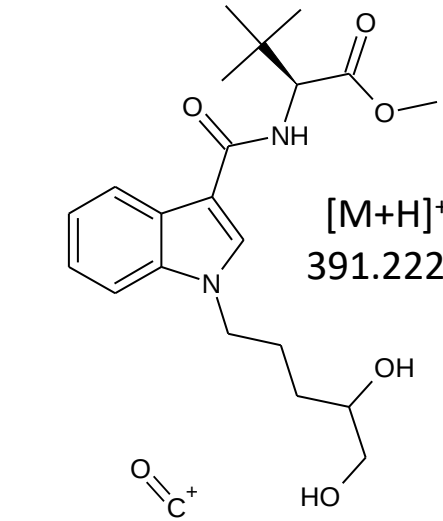


144.0444

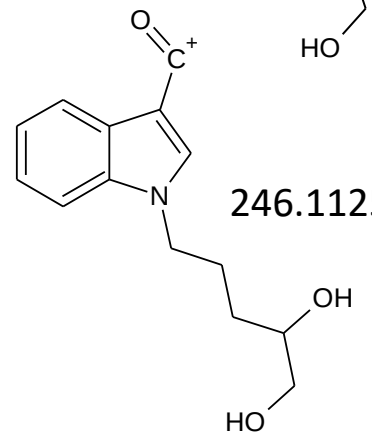
E2, Dihydrodiol formation, RT 7.05 min, *m/z* 391.2238



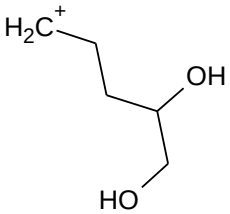




[M+H]+
391.2227

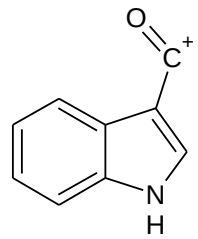


246.1125



103.0754
(not detected)

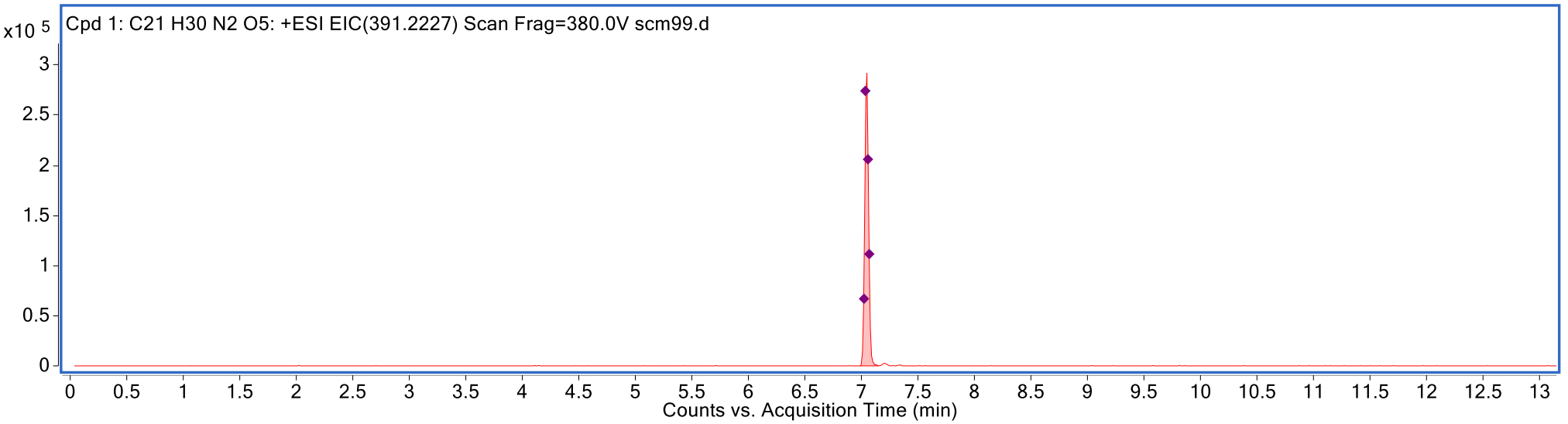
$-H_2O$

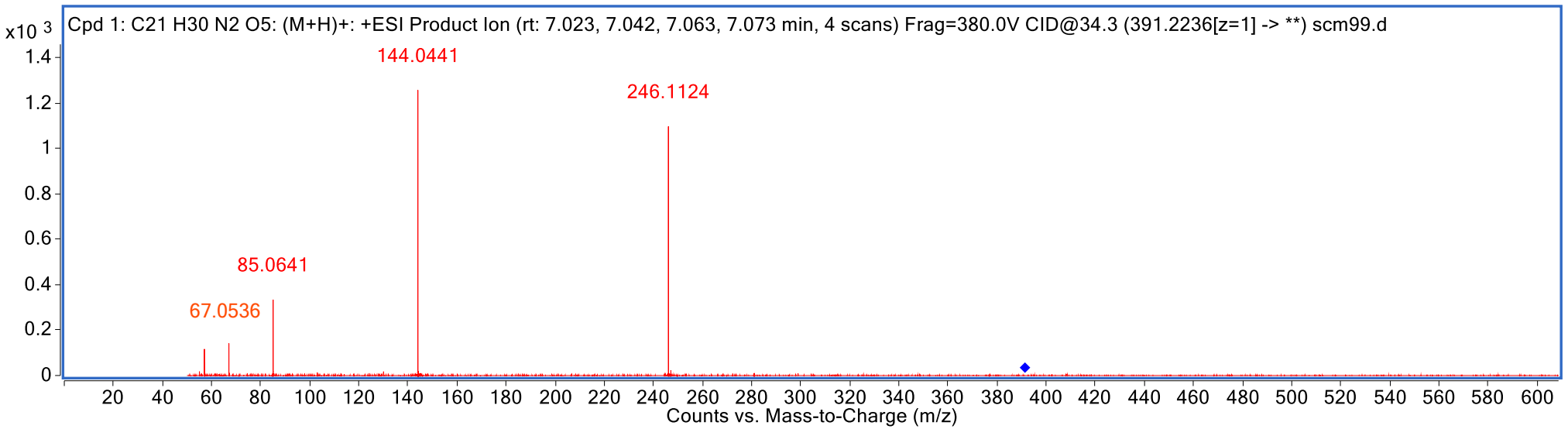


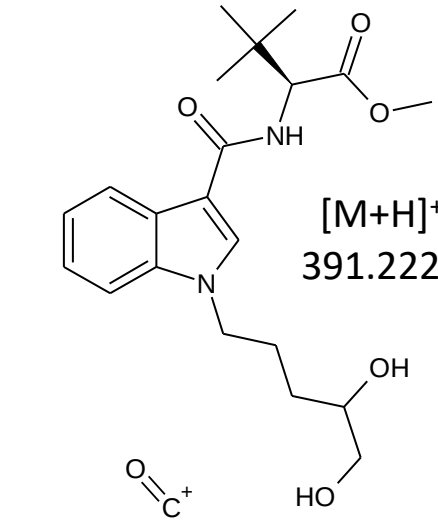
144.0444

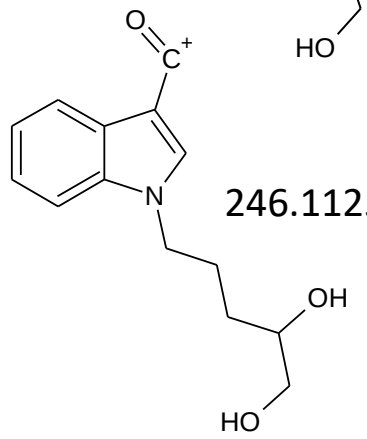
85.0648 $\xrightarrow{-H_2O}$ 67.0542

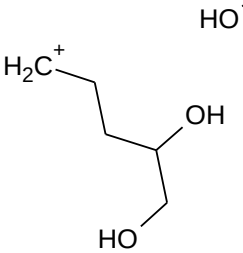
Dihydrodiol reference standard, RT 7.05 min, *m/z* 391.2236

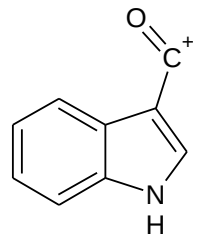










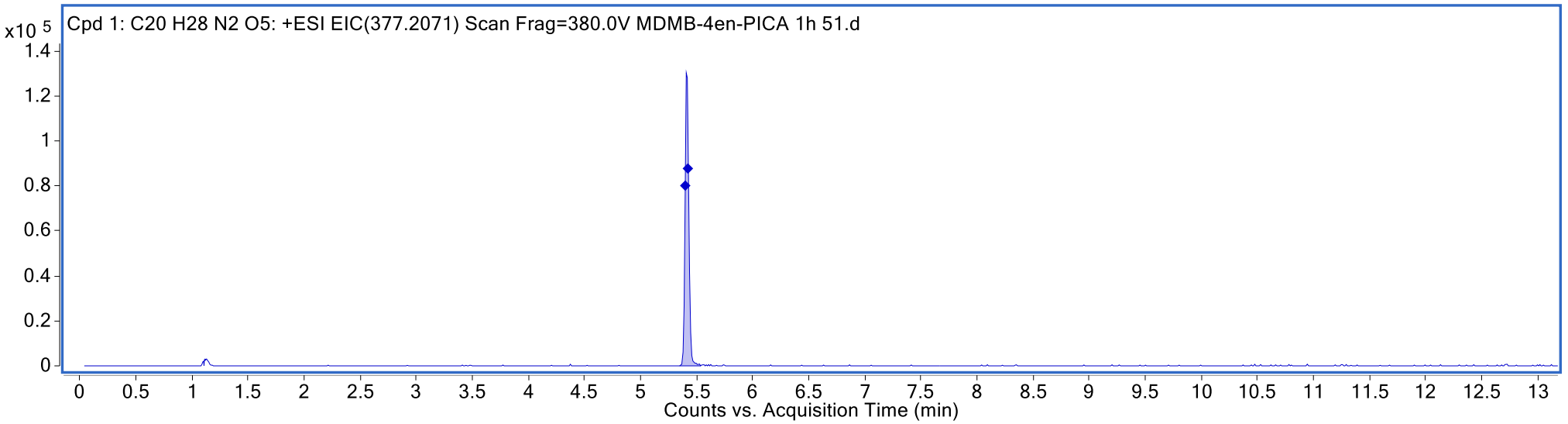


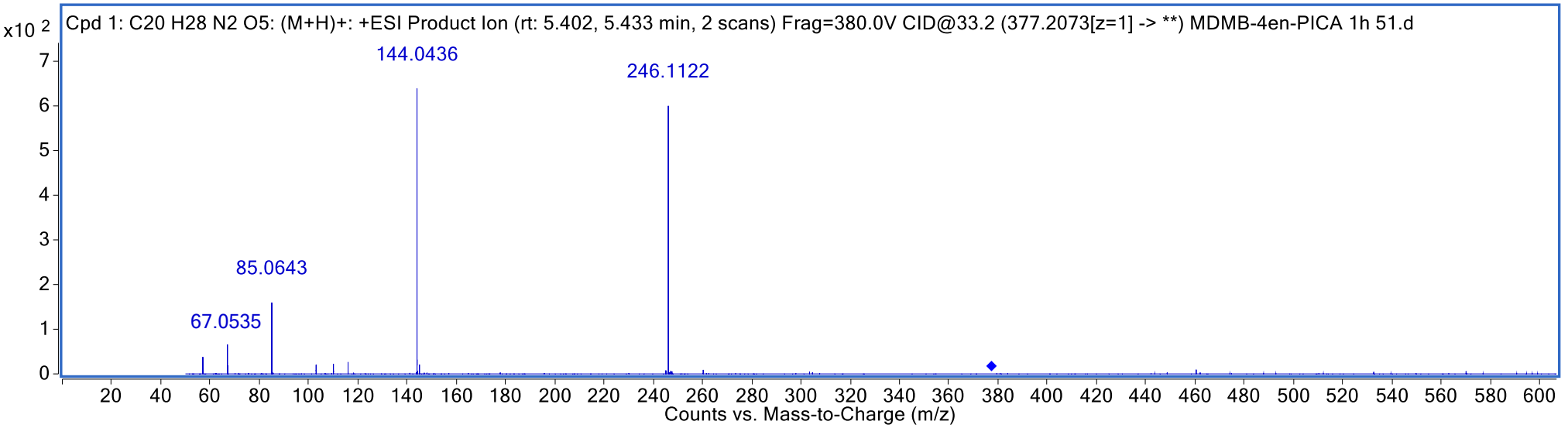
$-H_2O$

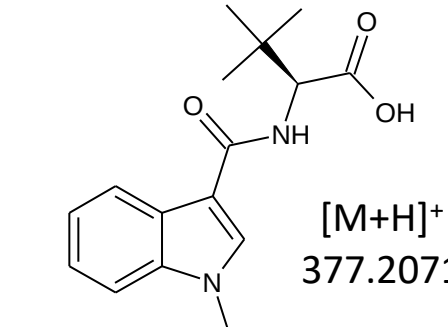
85.0648 → 67.0542

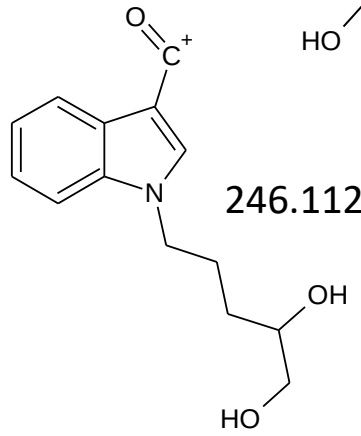
$-H_2O$

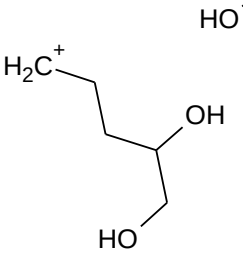
E3, Ester hydrolysis + dihydrodiol formation, RT 5.42 min, *m/z* 377.2072

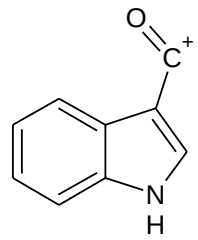










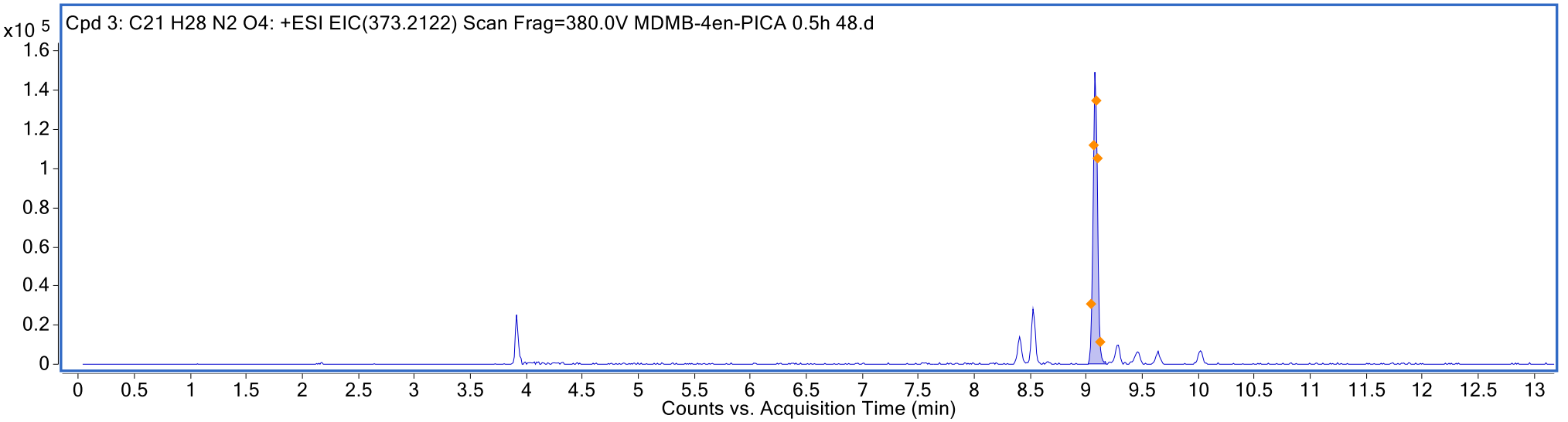


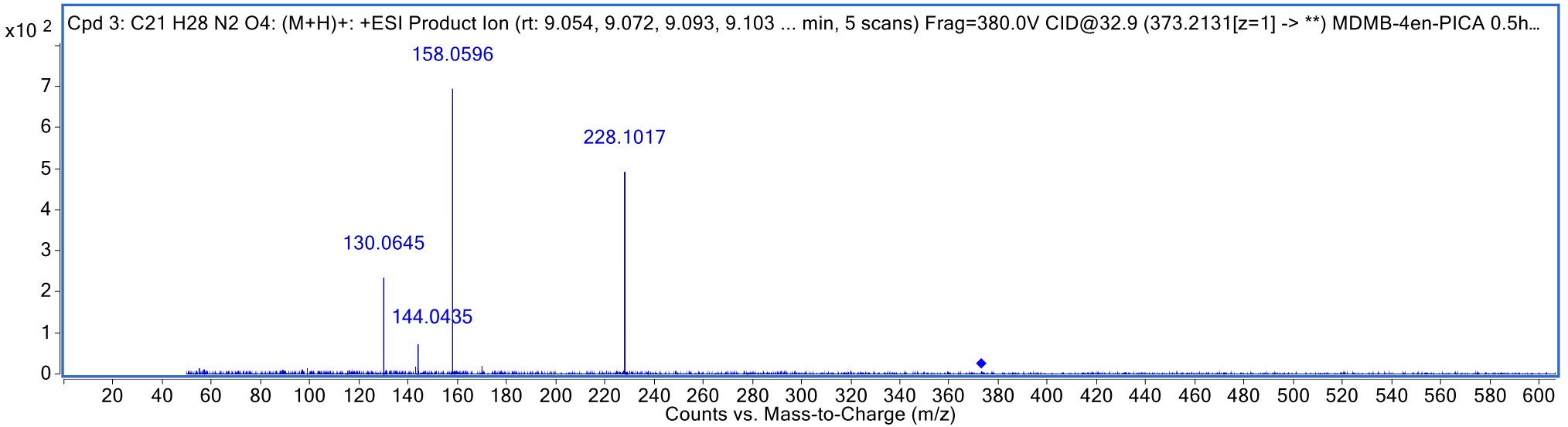
$-H_2O$

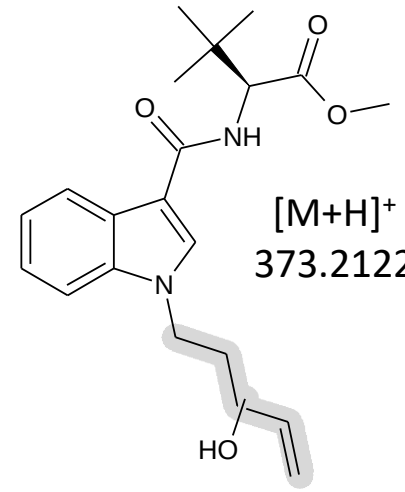
85.0648 → 67.0542

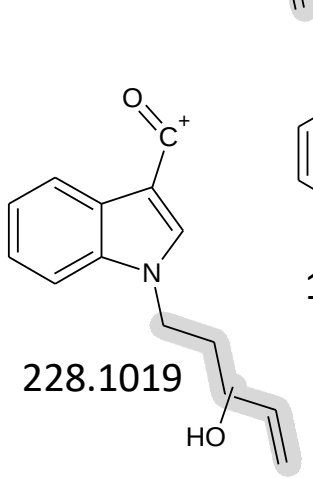
$-H_2O$

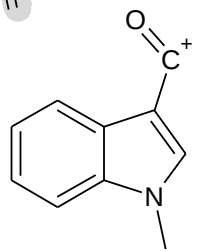
E4, Mono-hydroxylation (pentenyl tail), RT 9.08 min, *m/z* 373.2131

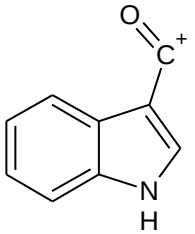


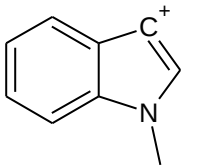




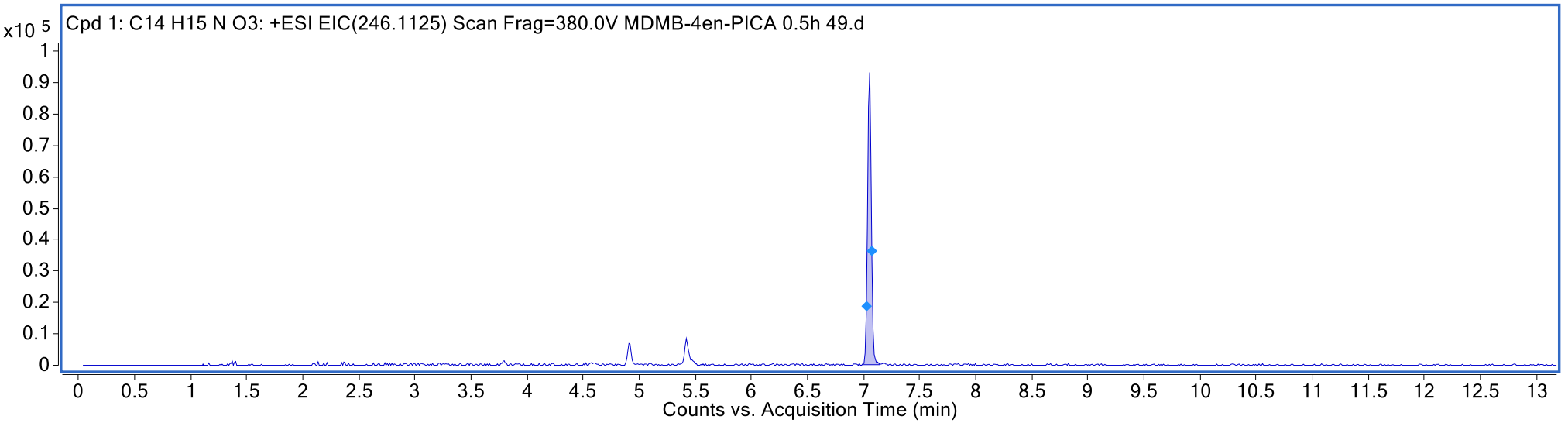


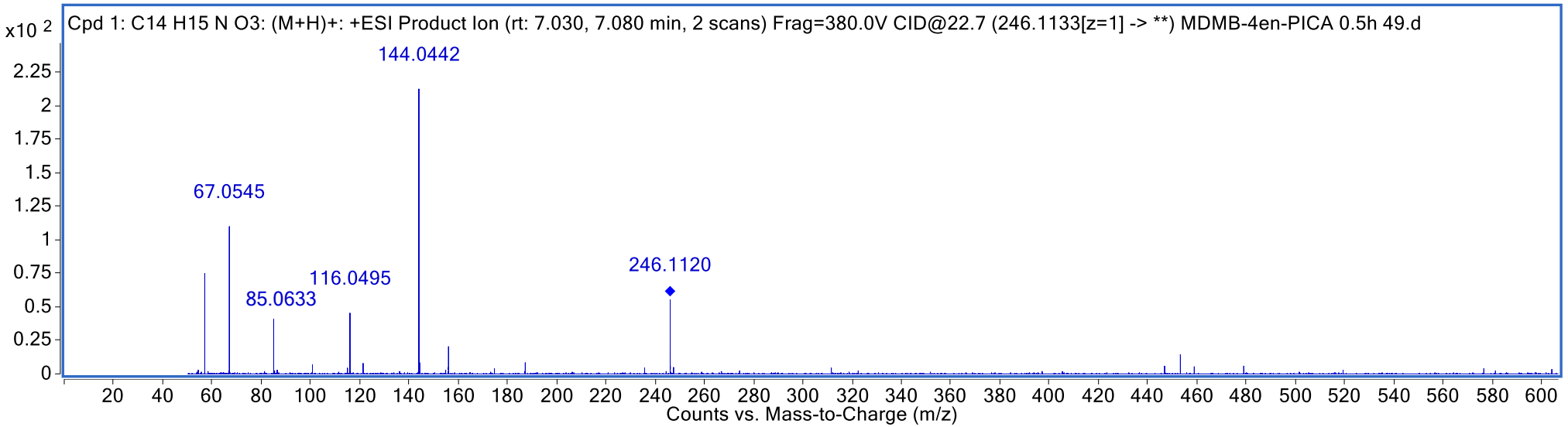


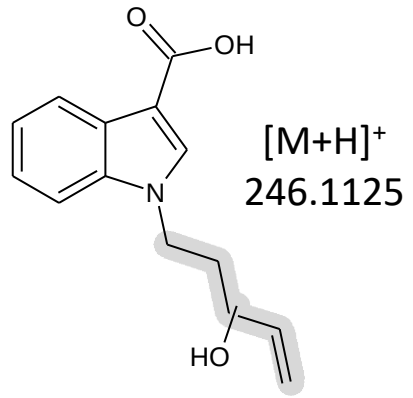




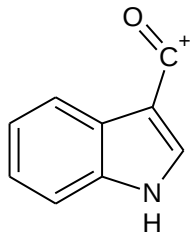
E5, Secondary amide hydrolysis + mono-hydroxylation (pentenyl tail), RT 7.05 min, *m/z* 246.1133



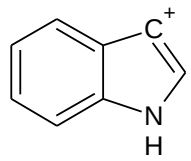




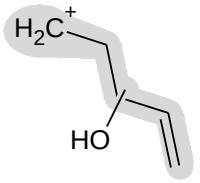
$[M+H]^+$
246.1125



144.0444

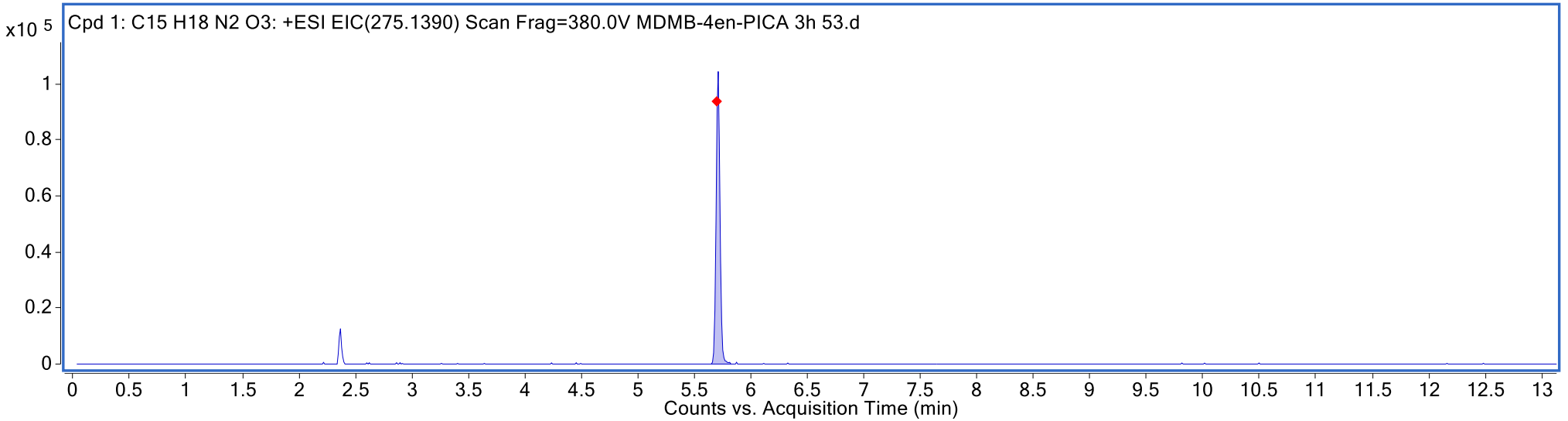


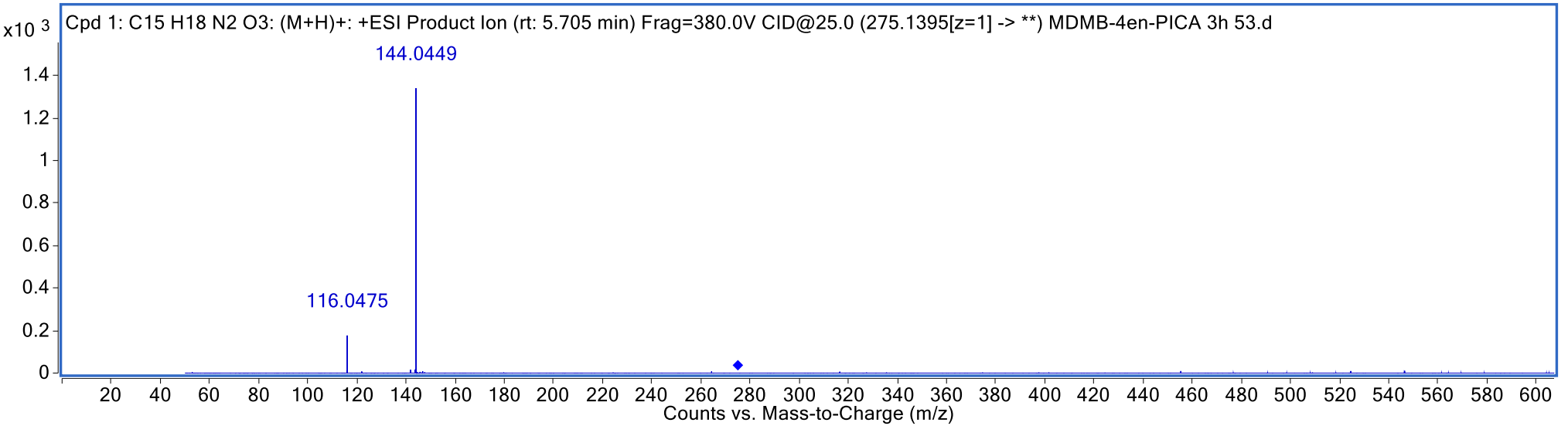
116.0495

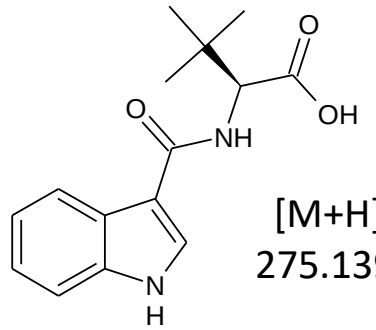


85.0648 $\xrightarrow{-H_2O}$ 67.0542

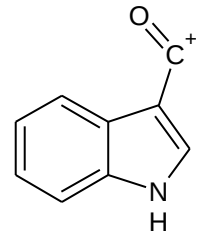
E6, Ester hydrolysis + *N*-dealkylation, RT 5.71 min, *m/z* 275.1396



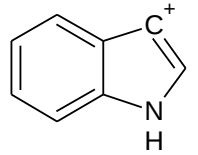




[M+H]⁺
275.1390

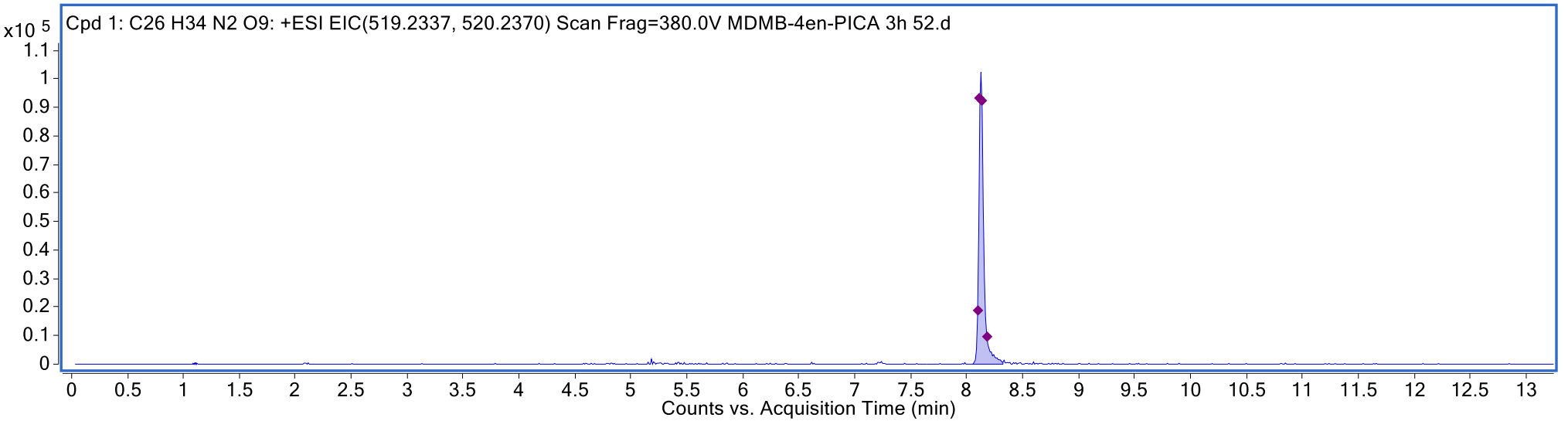


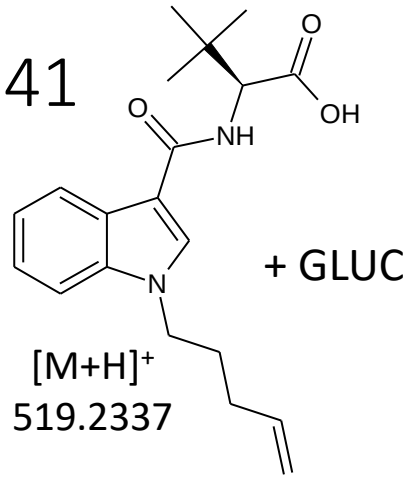
144.0444



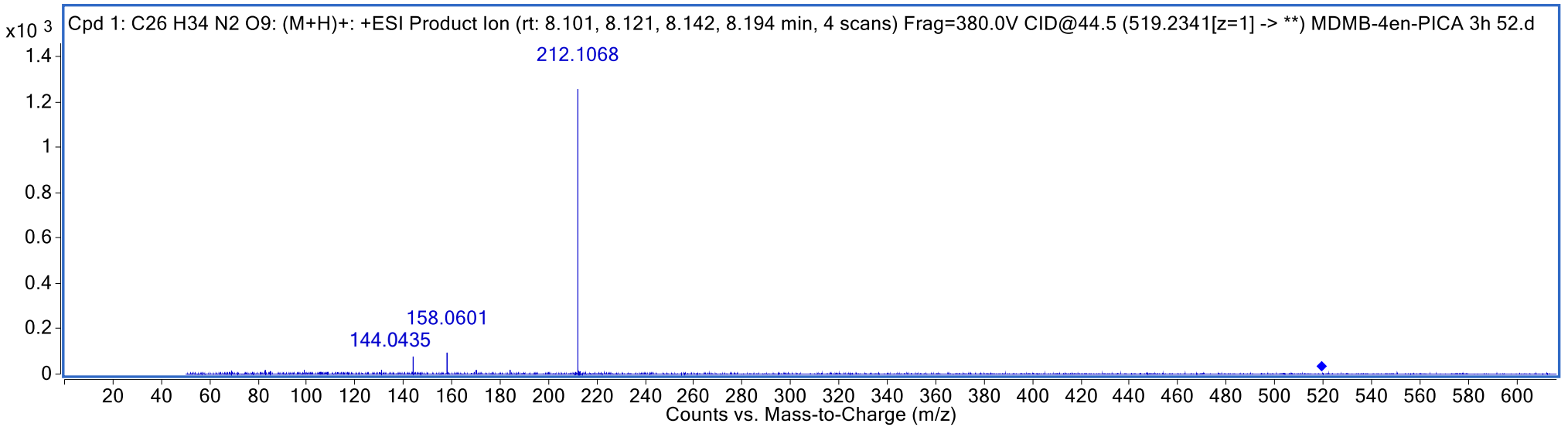
116.0495

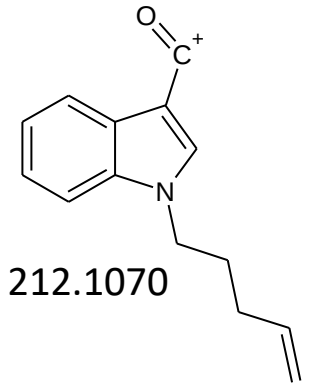
E7, Ester hydrolysis + glucuronidation, RT 8.13 min, *m/z* 519.2341



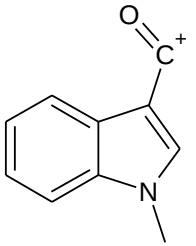


[M+H]+
519.2337

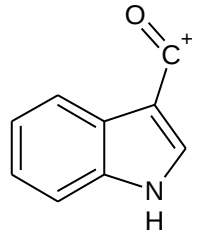




212.1070

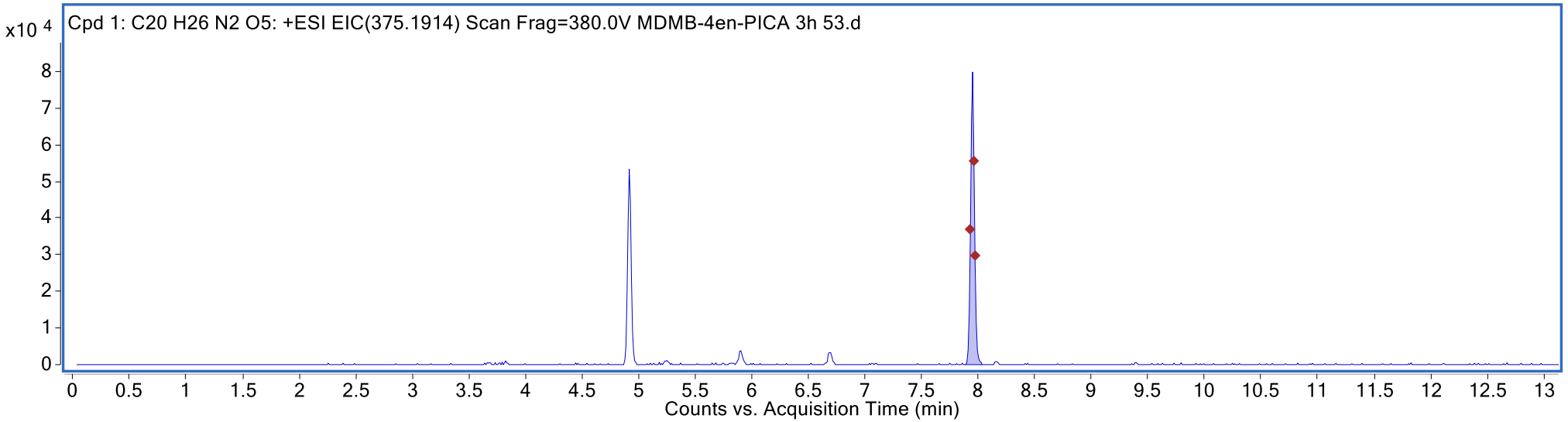


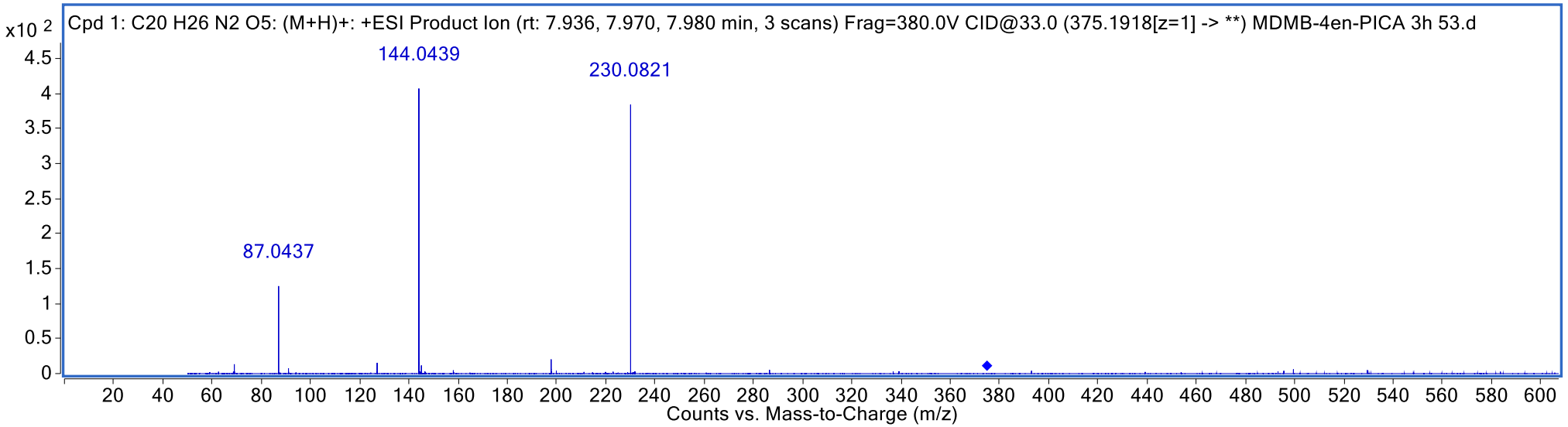
158.0600

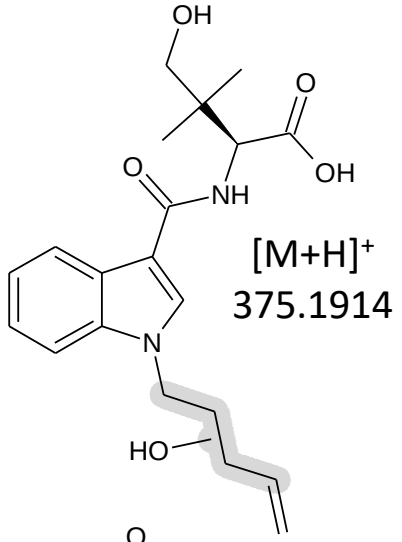


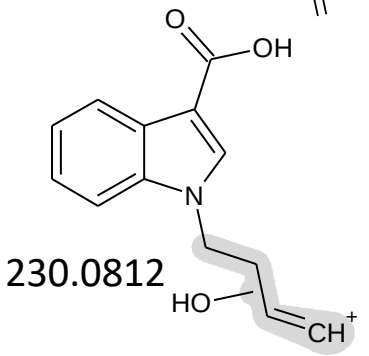
144.0444

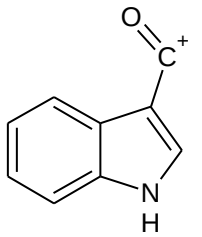
E8, Ester hydrolysis + di-hydroxylation (pentenyl tail + *tert*-butyl), RT 7.95 min, *m/z* 375.1917

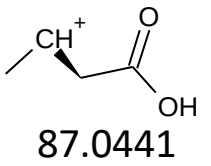




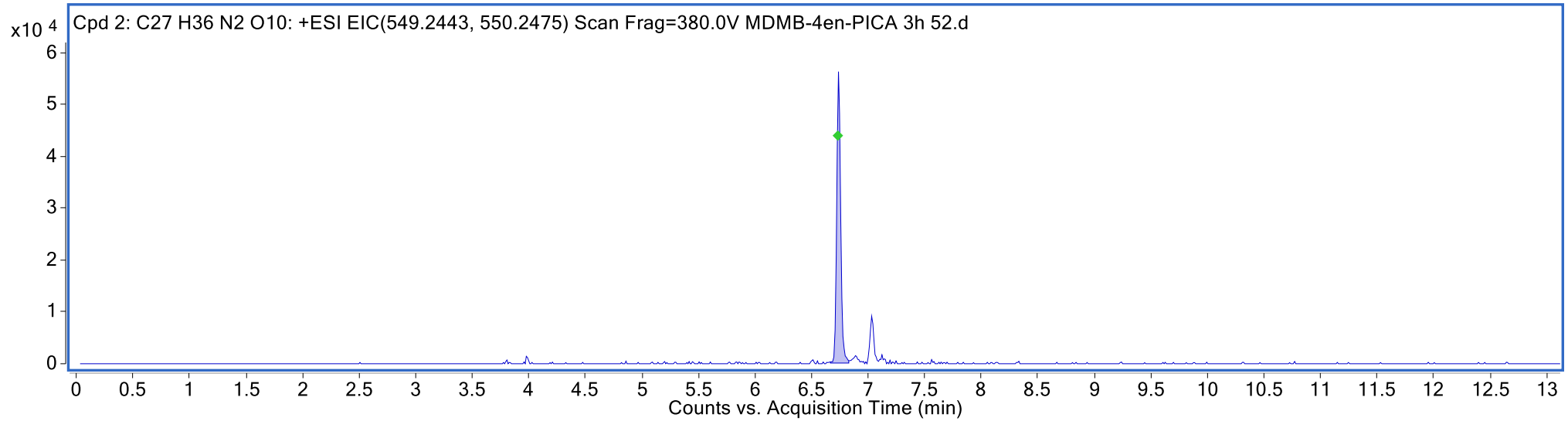


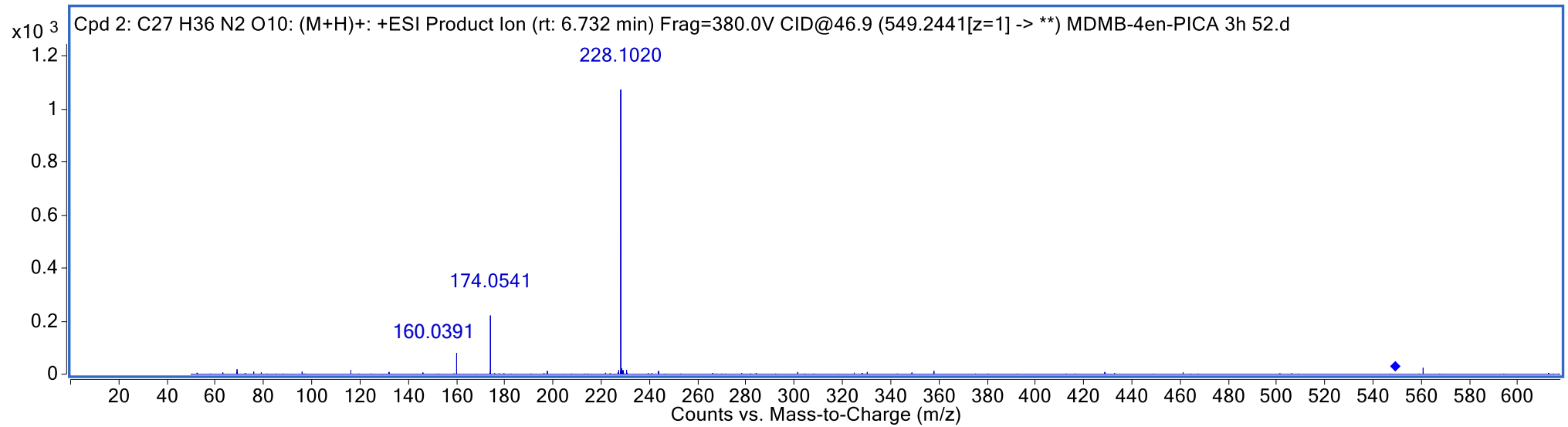


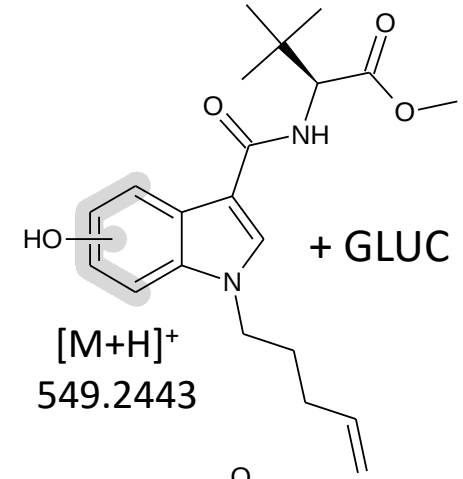


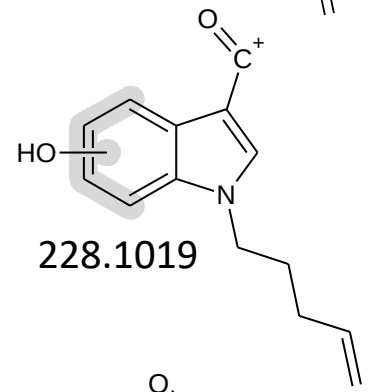


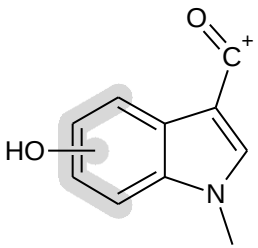
E9, Mono-hydroxylation + glucuronidation (indole core), RT 6.74 min, *m/z* 549.2434

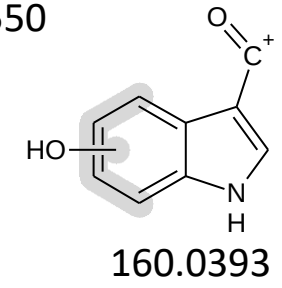




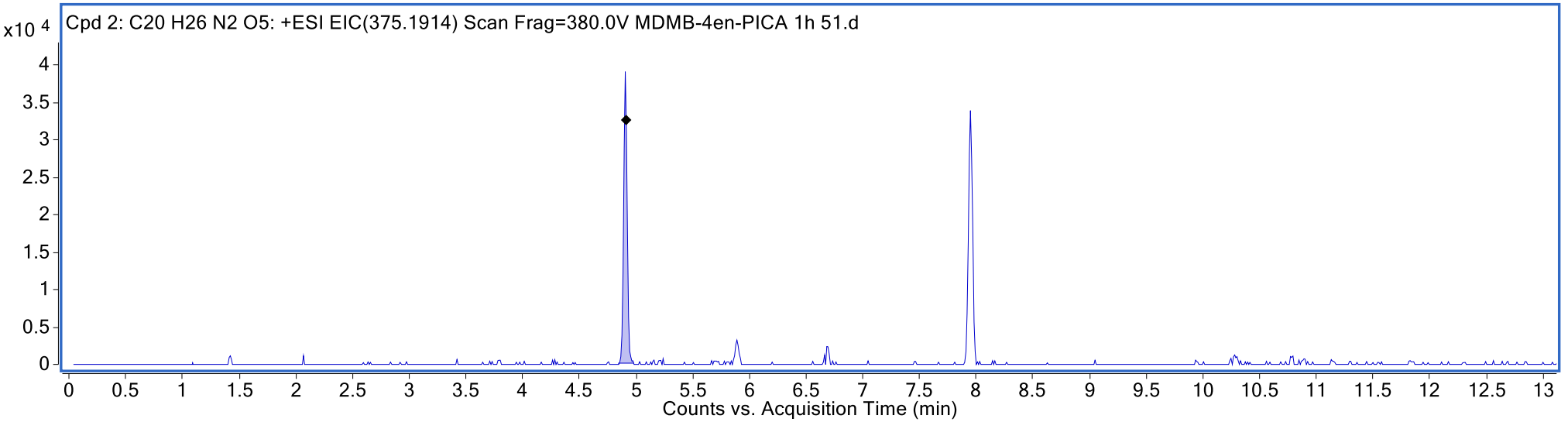


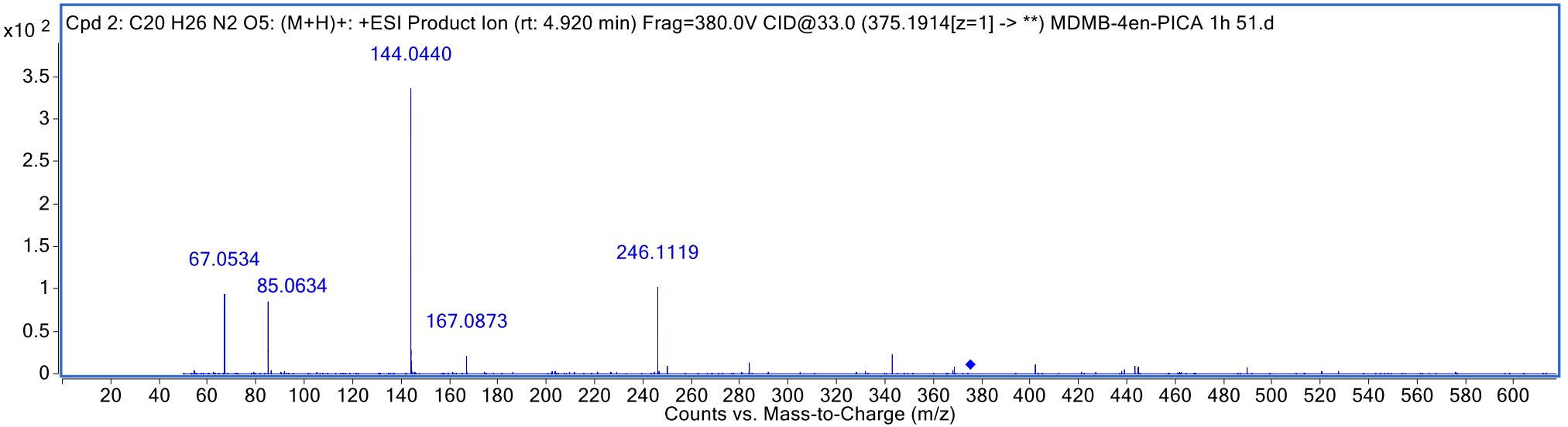


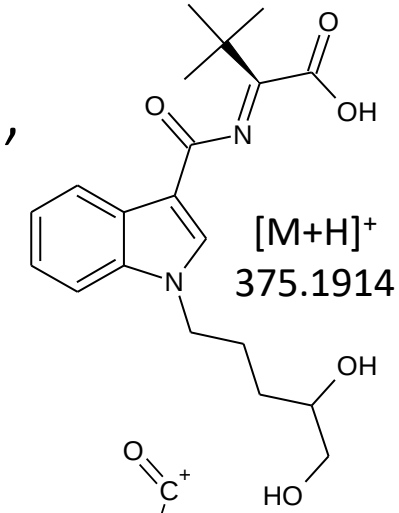


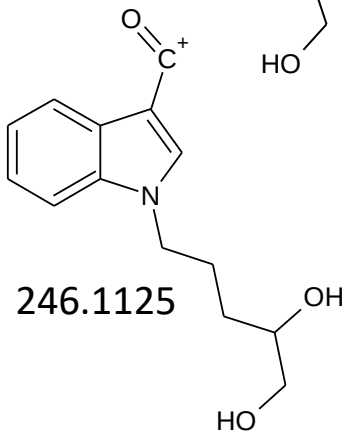


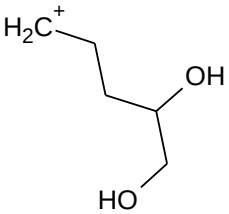
E10, Ester hydrolysis + dihydrodiol formation + dehydrogenation, RT 4.91 min, *m/z* 375.1916

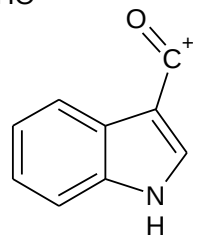








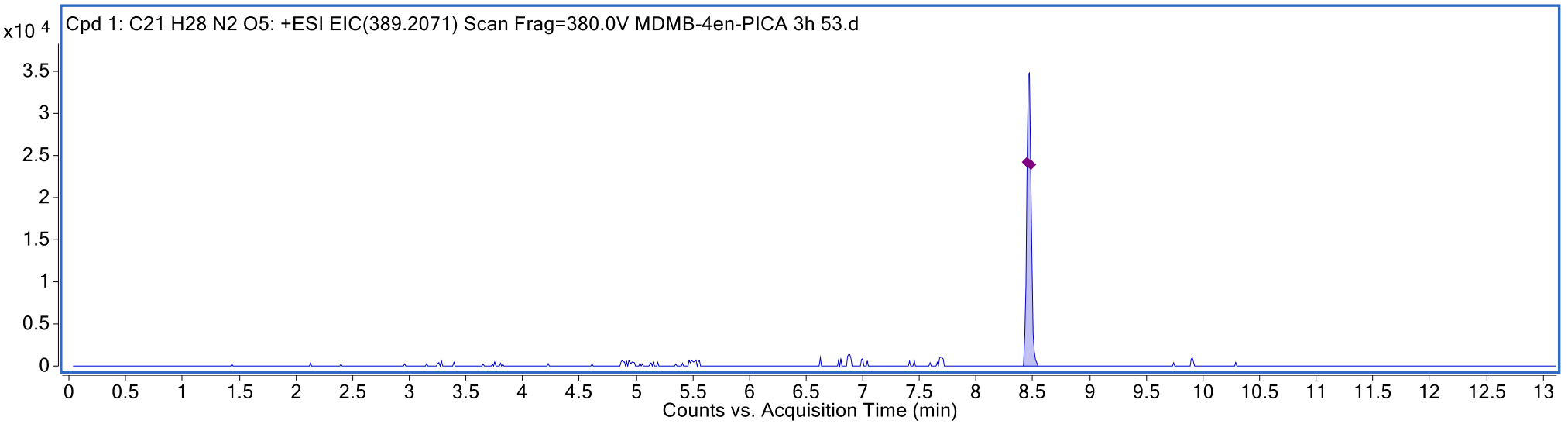


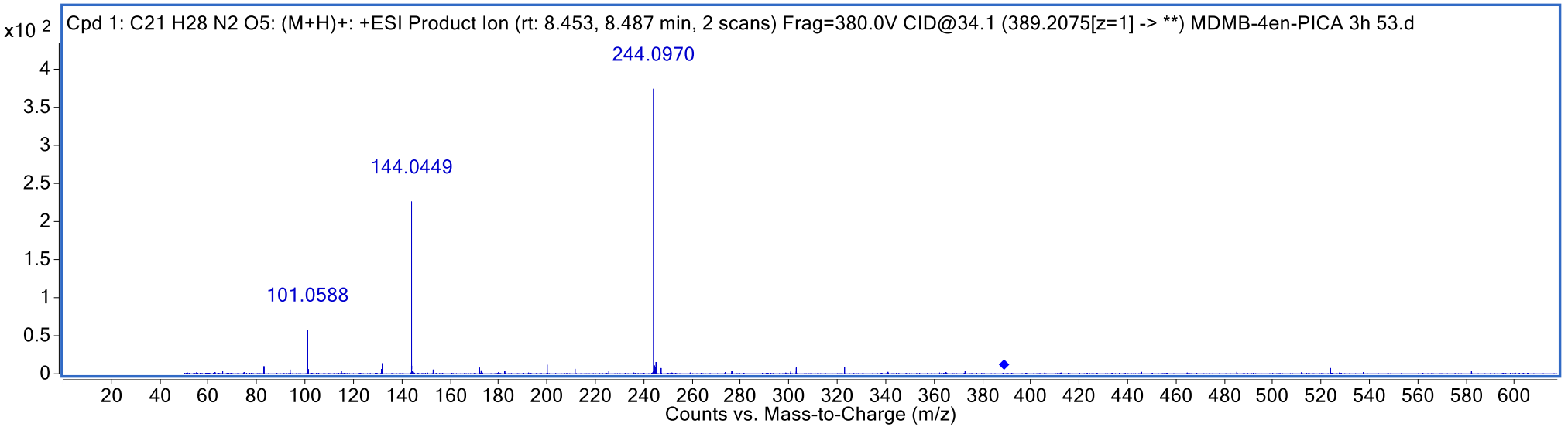


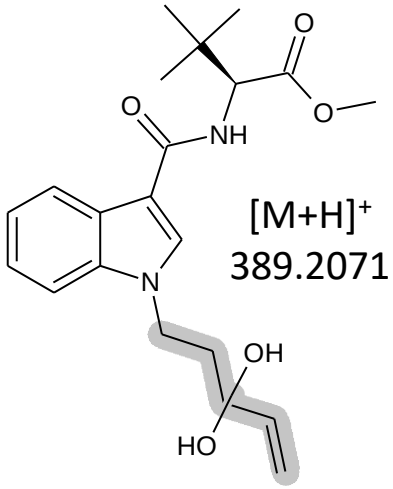
$-H_2O$

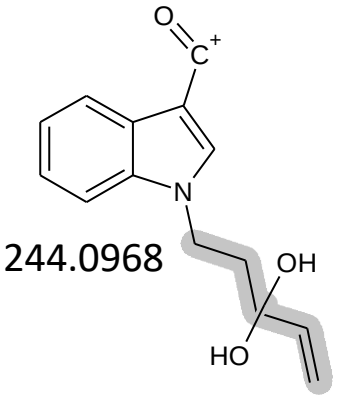
85.0648 → 67.0542 ($-H_2O$)

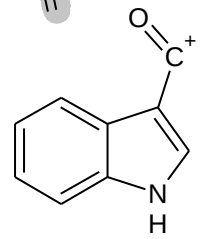
E11, Di-hydroxylation (pentenyl tail), RT 8.47 min, *m/z* 389.2077

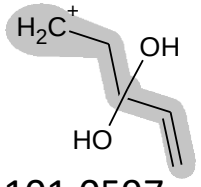




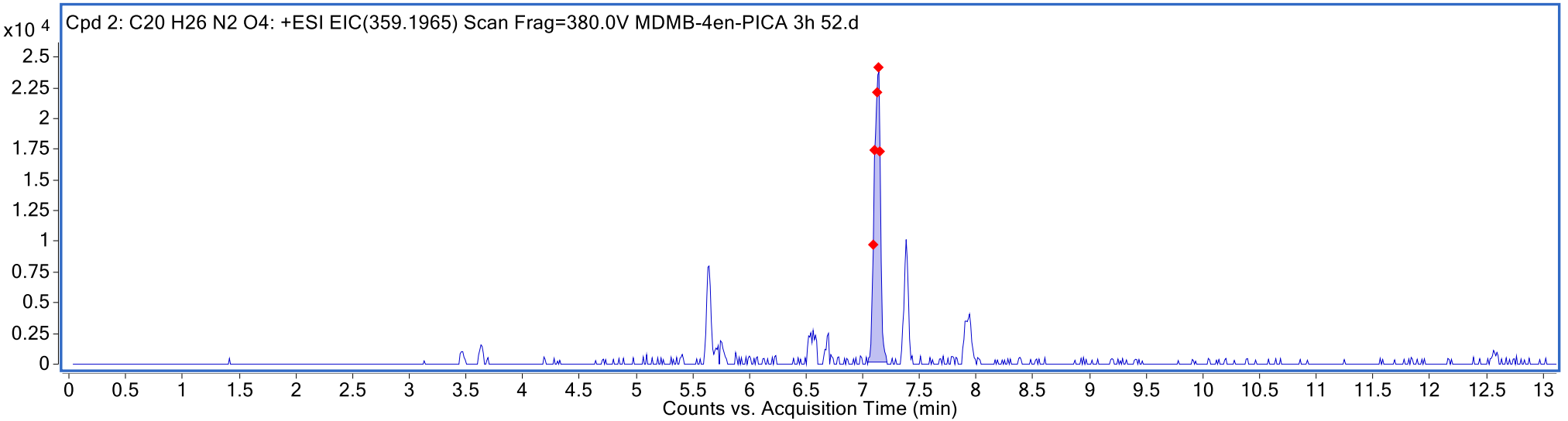


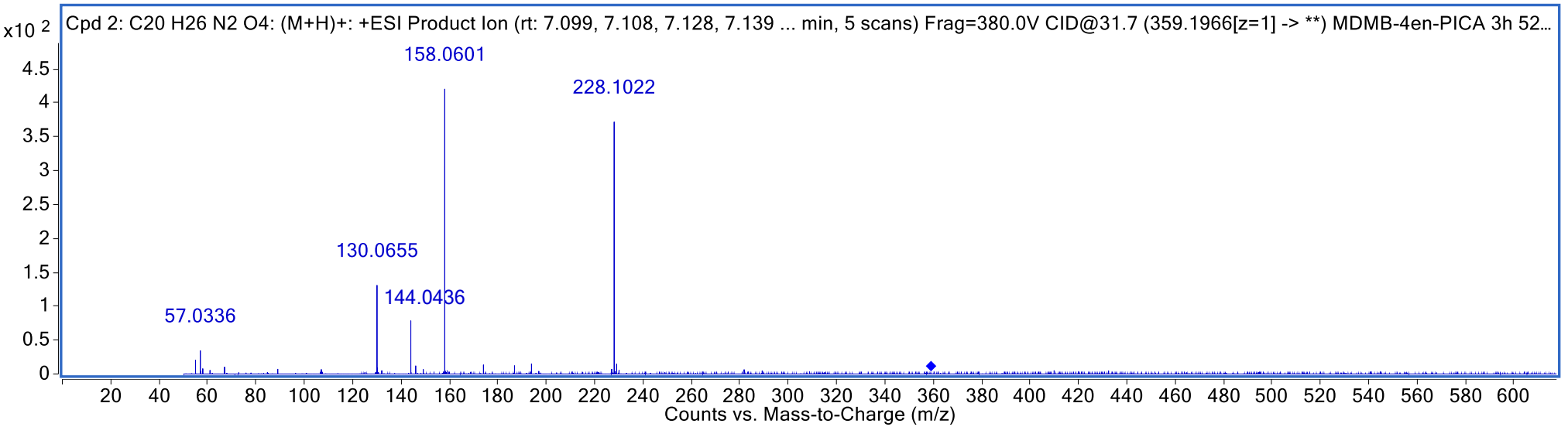


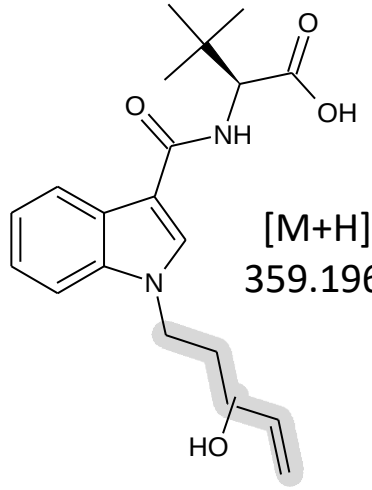




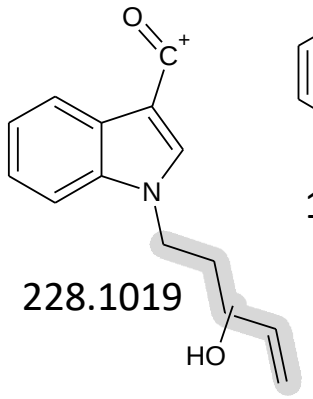
E12, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 7.14 min, *m/z* 359.1966



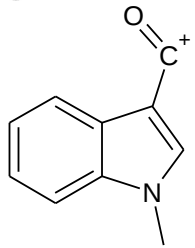




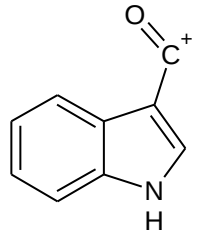
$[M+H]^+$
359.1965



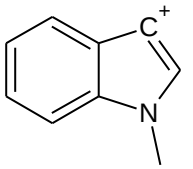
228.1019



158.0600

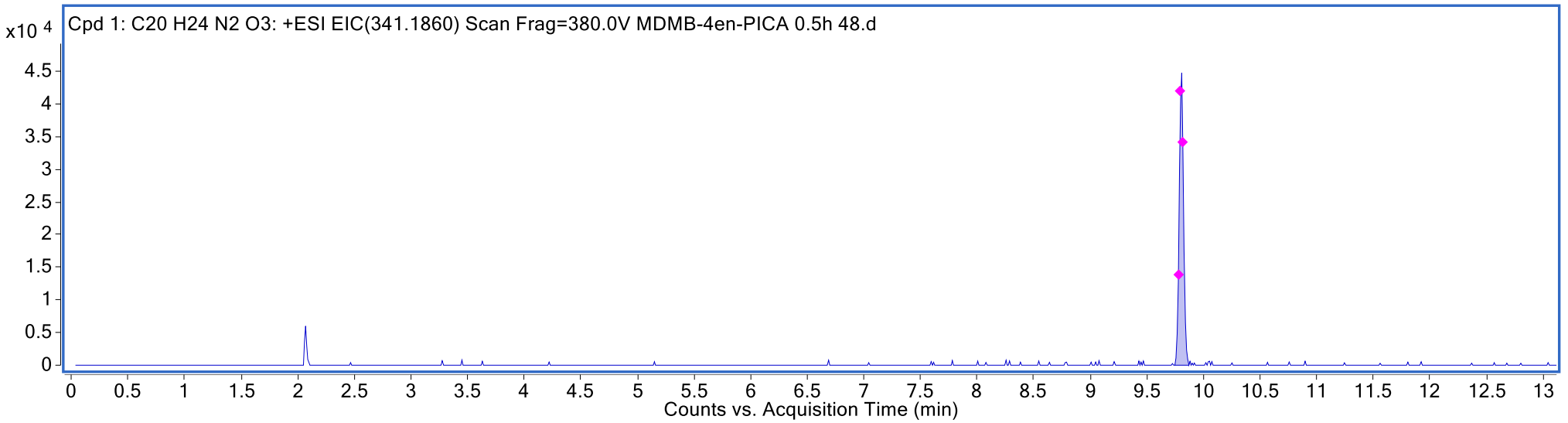


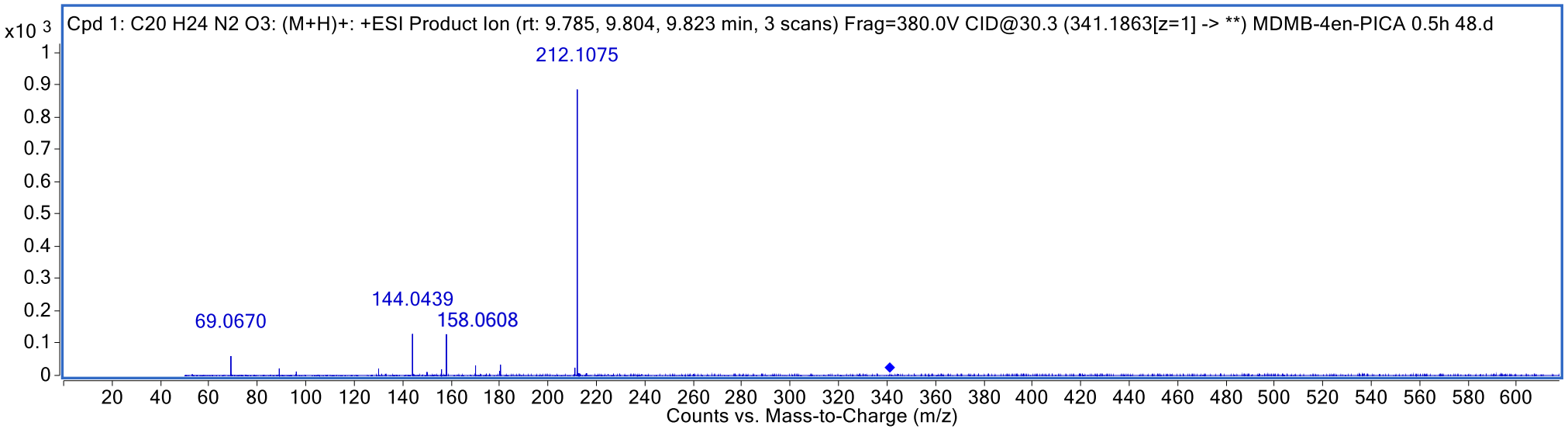
144.0444

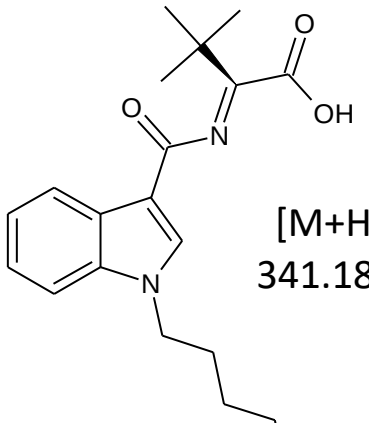


130.0651

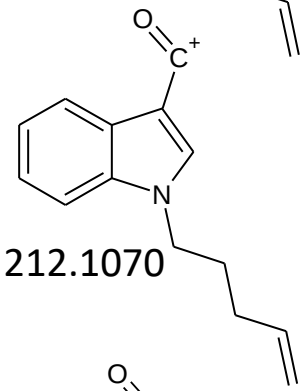
E13, Ester hydrolysis + dehydrogenation, RT 9.81 min, *m/z* 341.1863



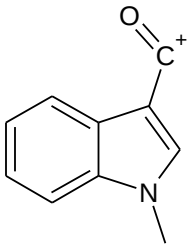




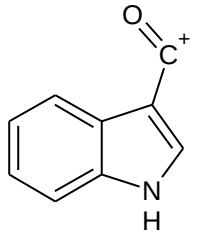
[M+H]+
341.1860



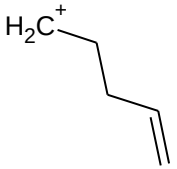
212.1070



158.0600

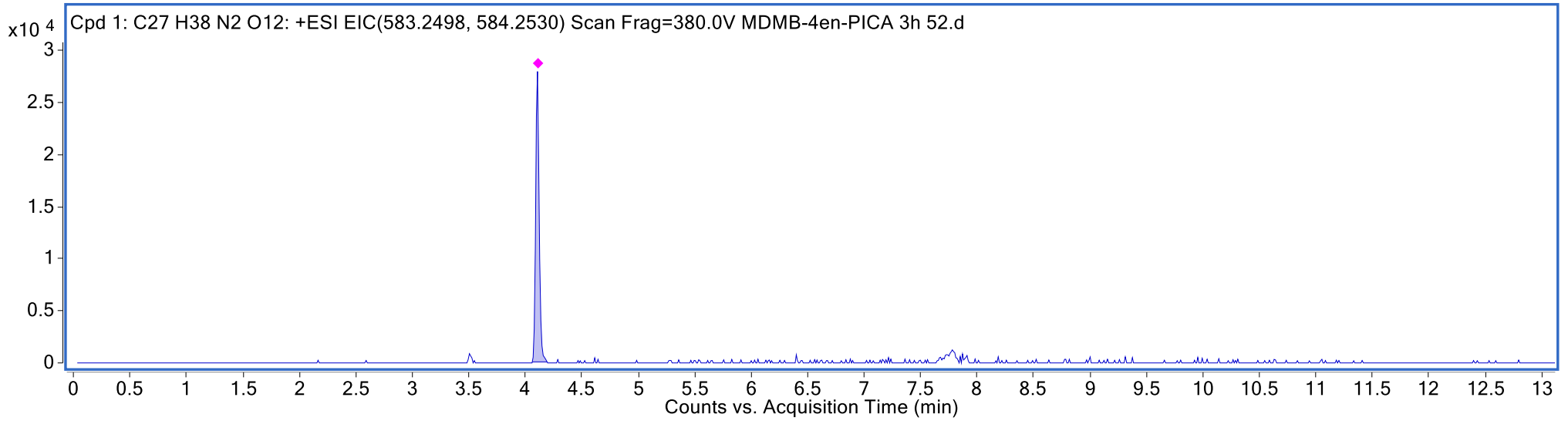


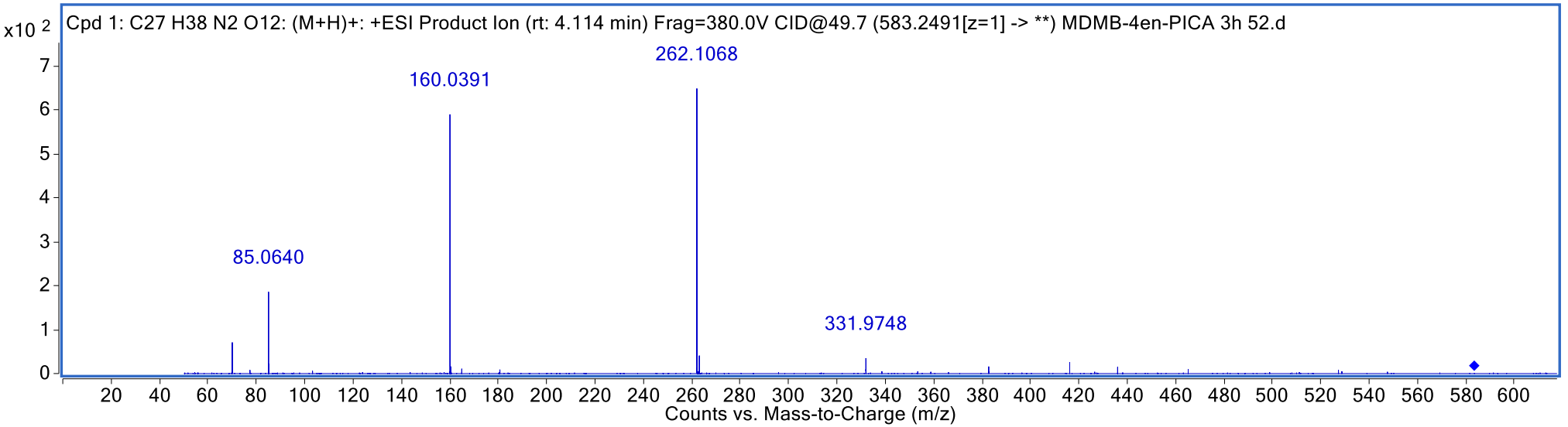
144.0444

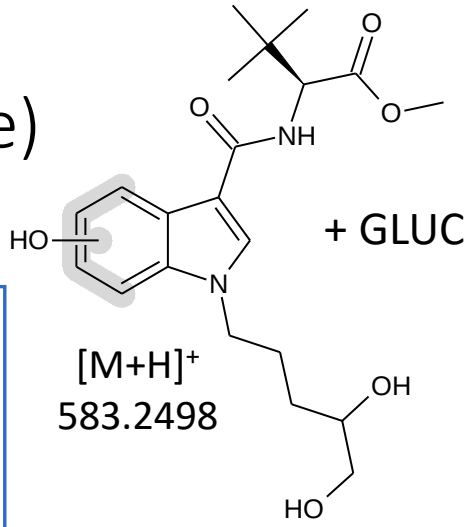


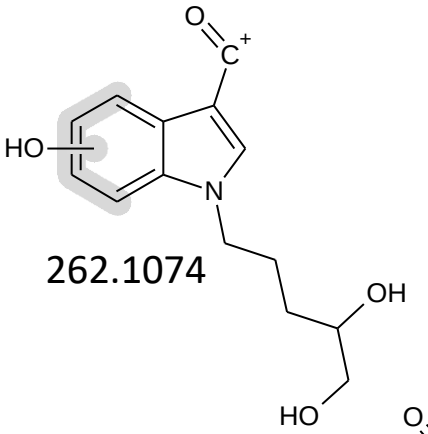
69.0699

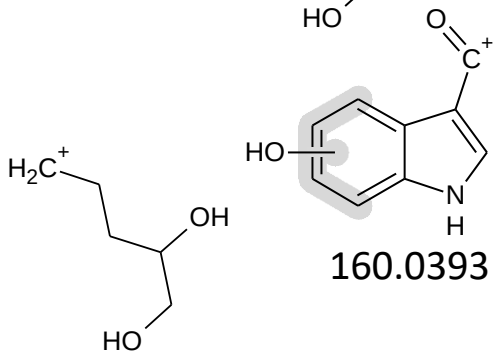
E14, Dihydrodiol formation + mono-hydroxylation (indole core) + glucuronidation, RT 4.11 min, *m/z* 583.2490



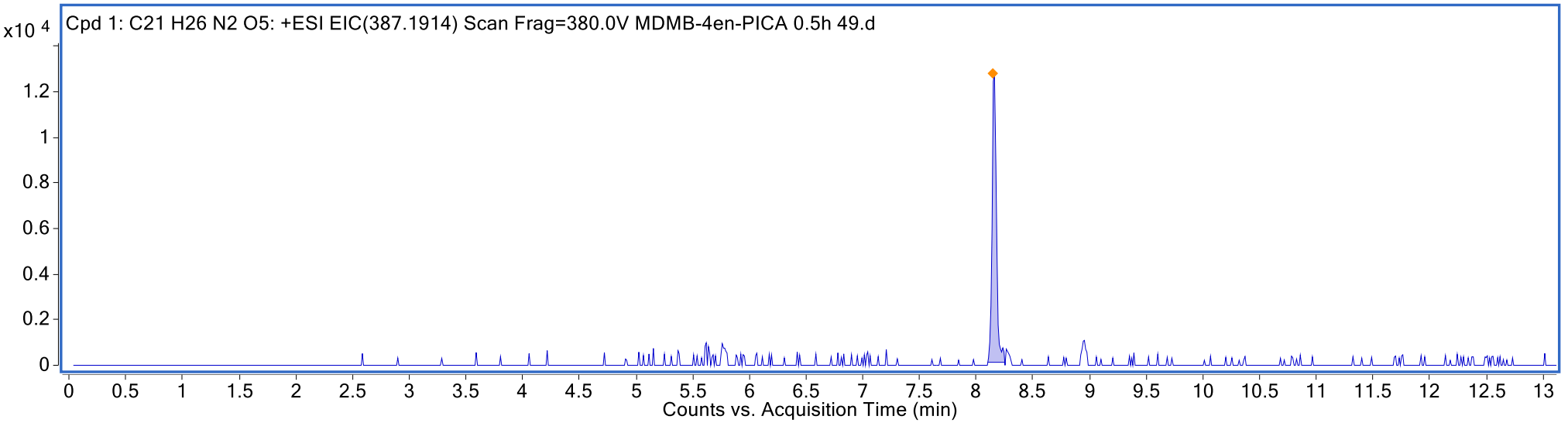


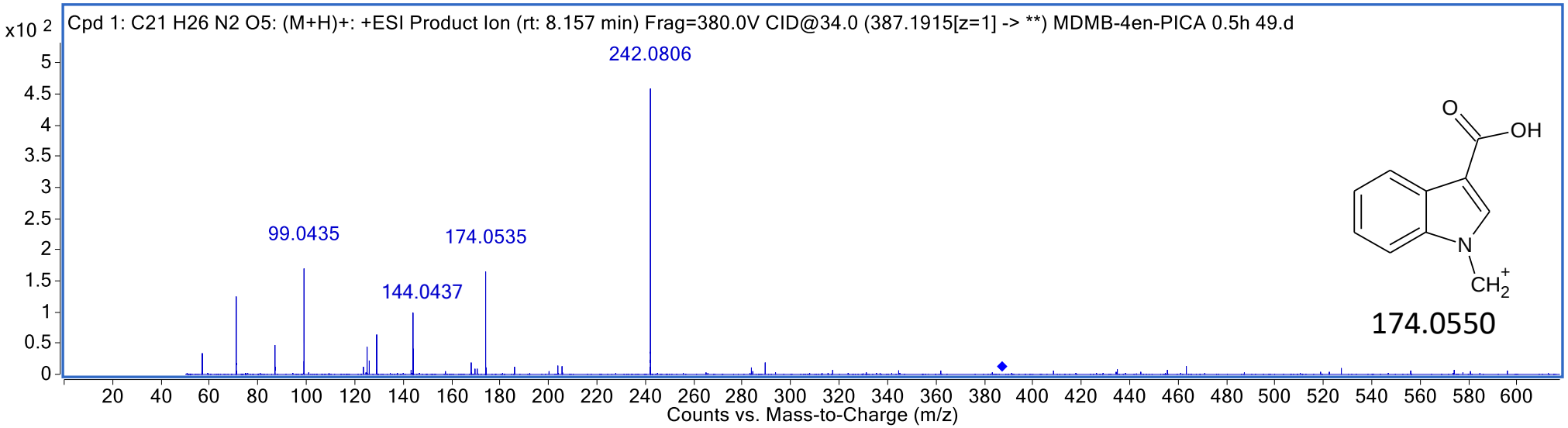


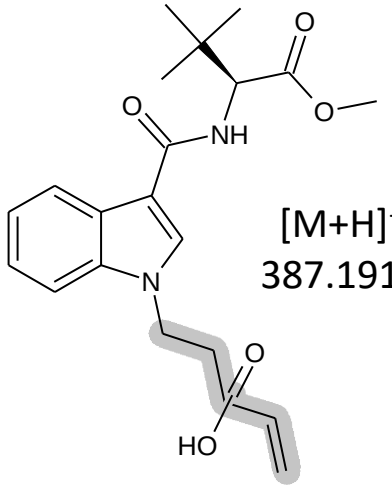




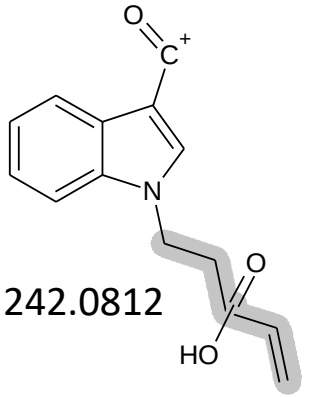
E15, Ketone formation + mono-hydroxylation (pentenyl tail), RT 8.16 min, *m/z* 387.1915



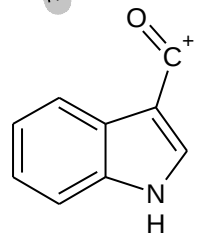




$[M+H]^+$
387.1914

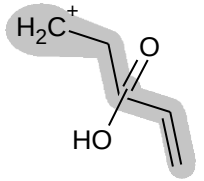


242.0812



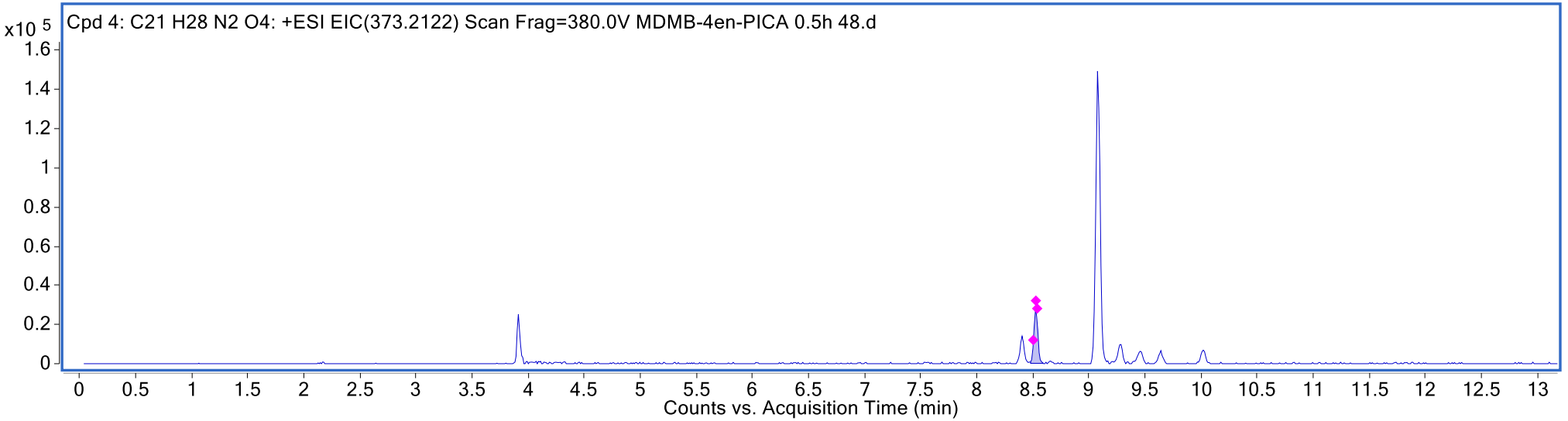
144.0444

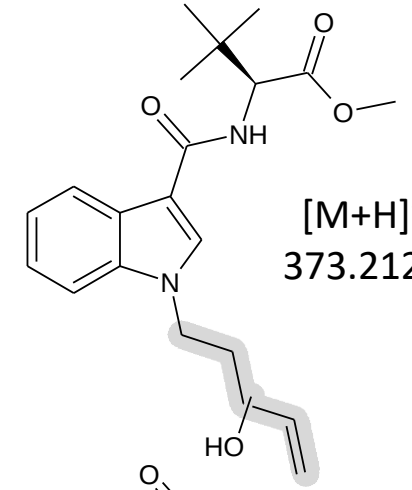
174.0550

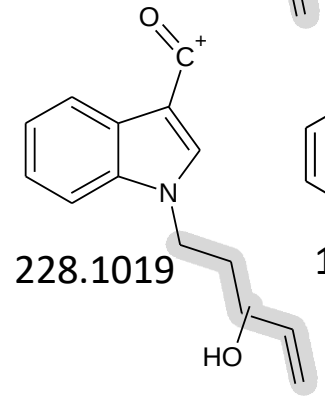


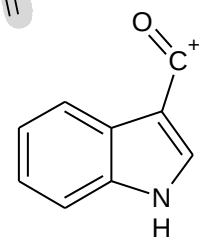
99.0441

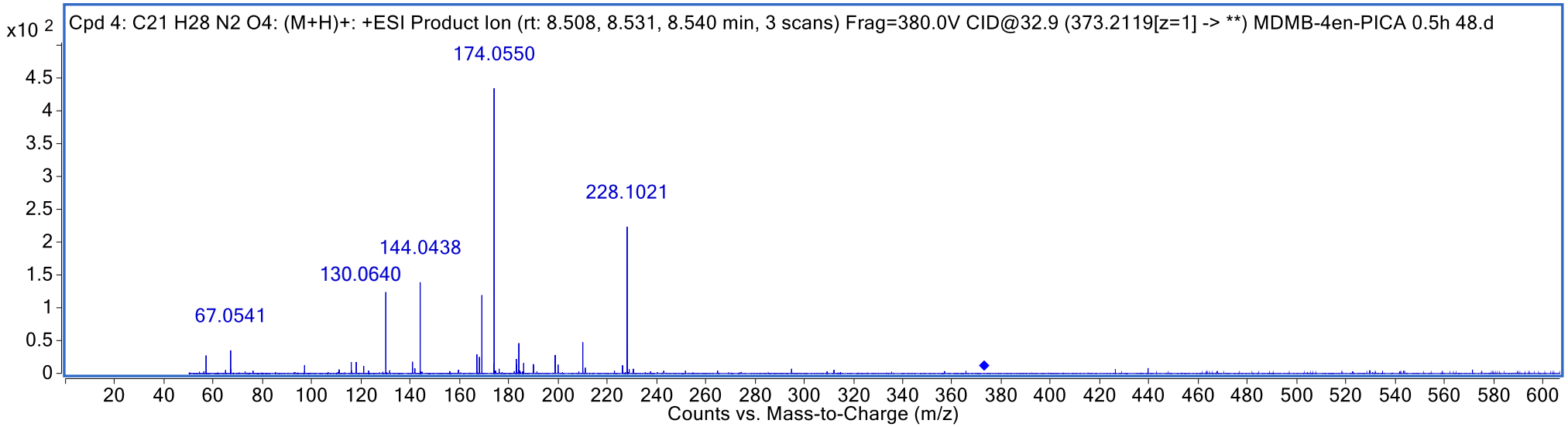
E16, Mono-hydroxylation (pentenyl tail), RT 8.53 min, *m/z* 373.2117

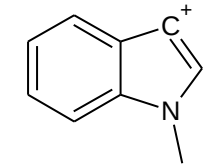


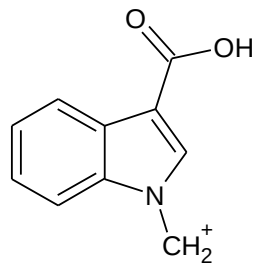


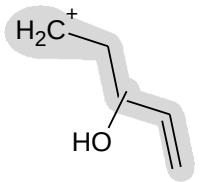




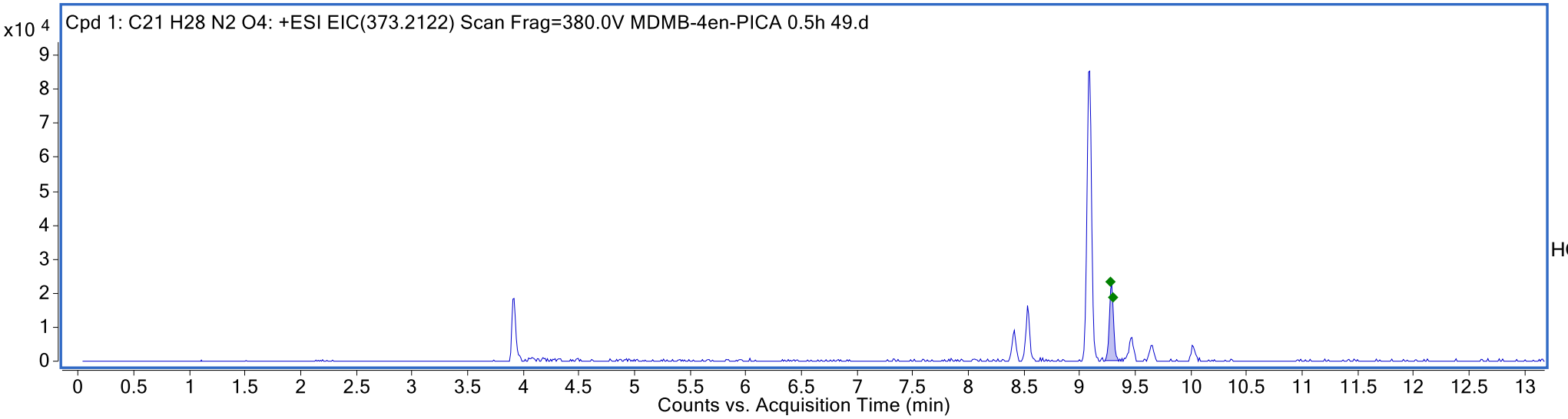


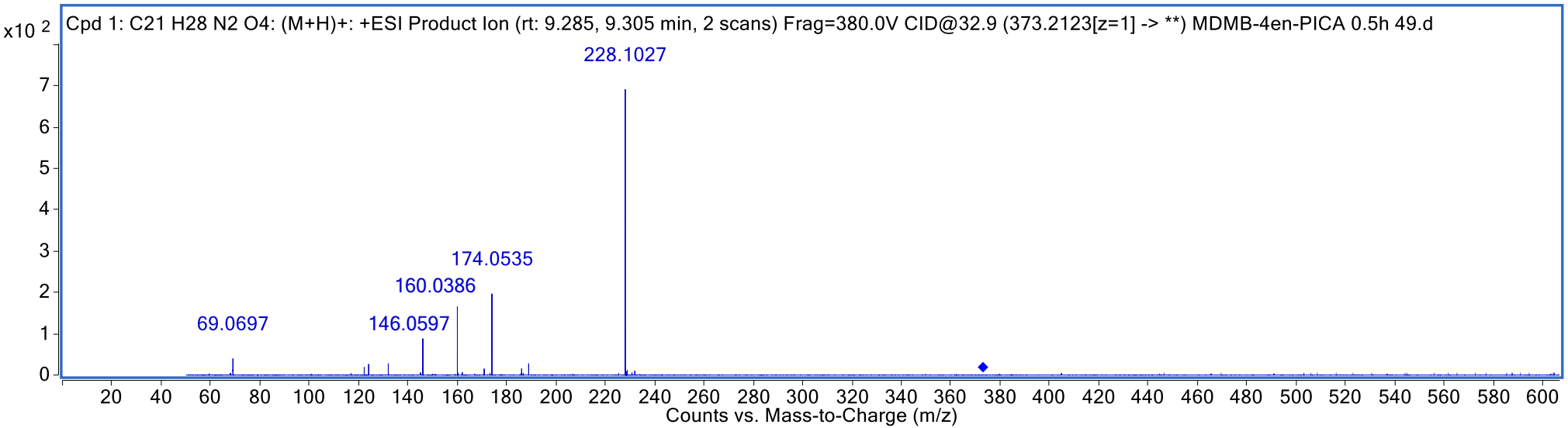


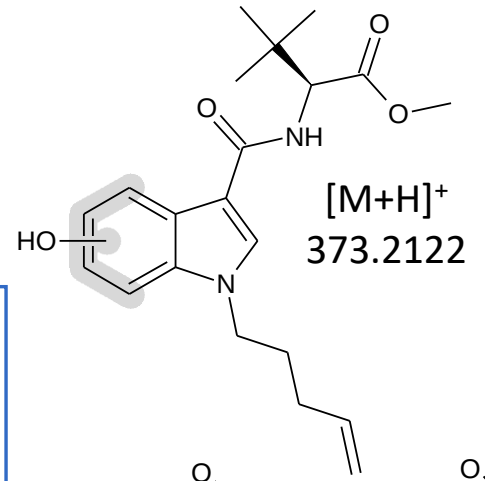


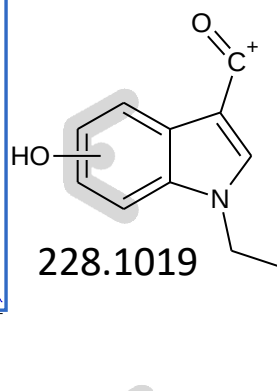


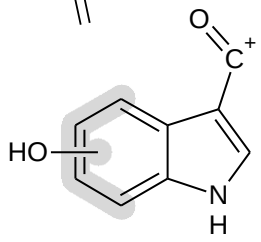
E17, Mono-hydroxylation (indole core), RT 9.29 min, *m/z* 373.2121

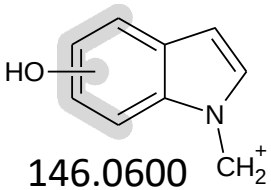


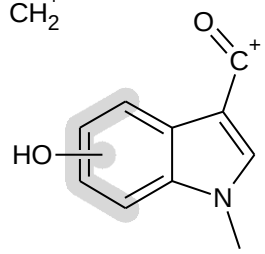


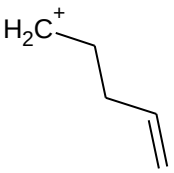




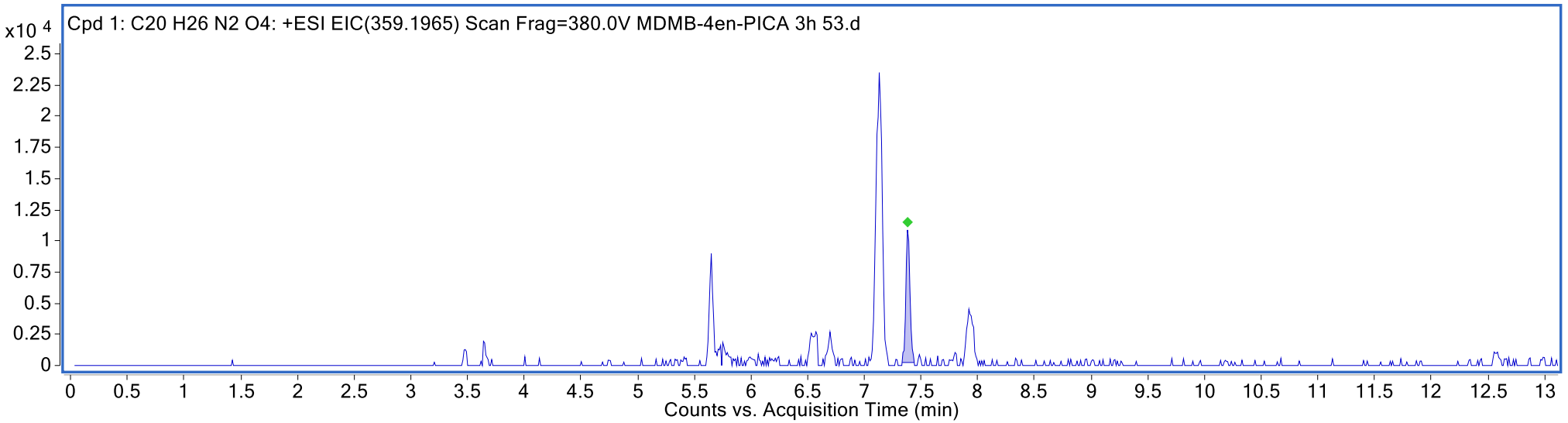


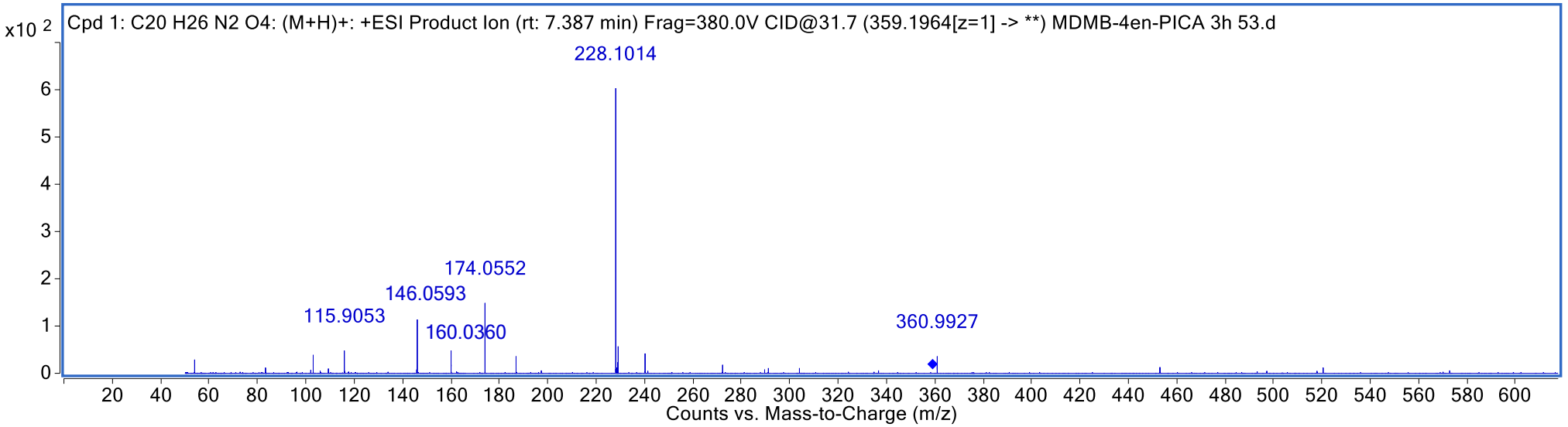


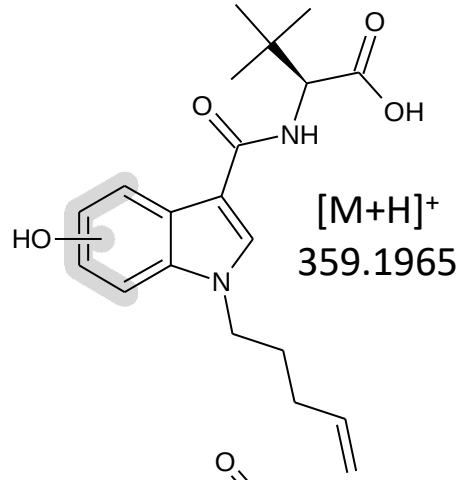


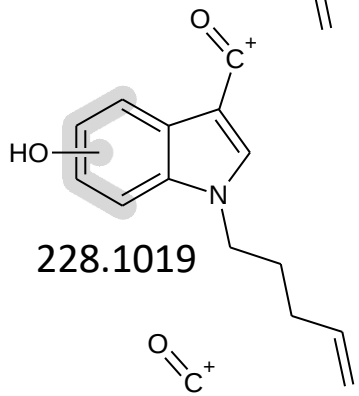


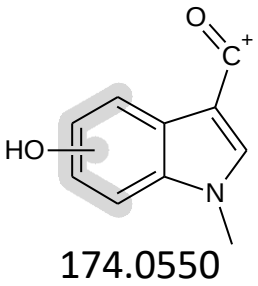
E18, Ester hydrolysis + mono-hydroxylation (indole core), RT 7.38 min, *m/z* 359.1963

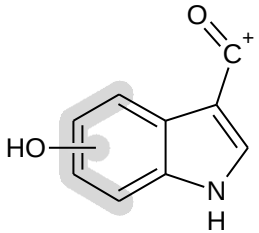


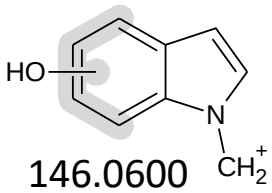




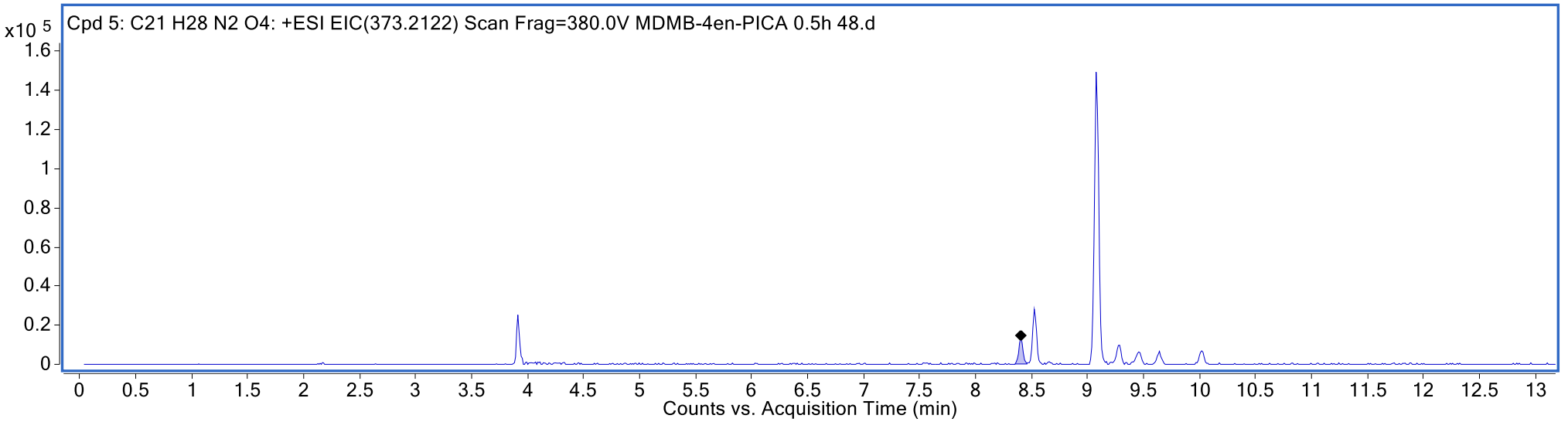


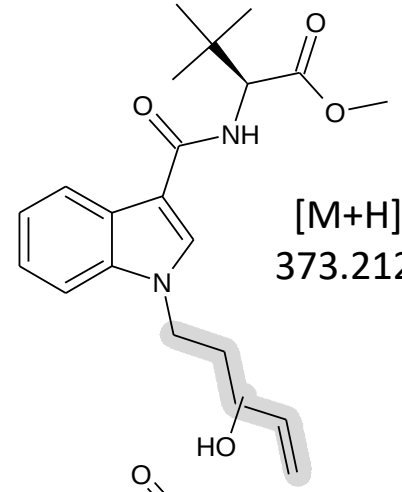




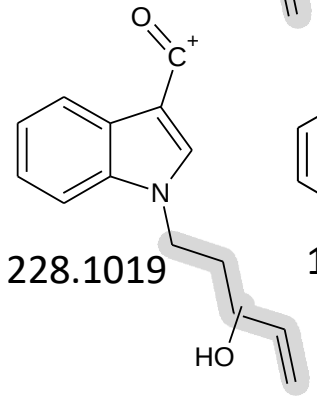


E19, Mono-hydroxylation (pentenyl tail), RT 8.41 min, *m/z* 373.2125

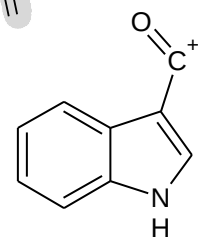




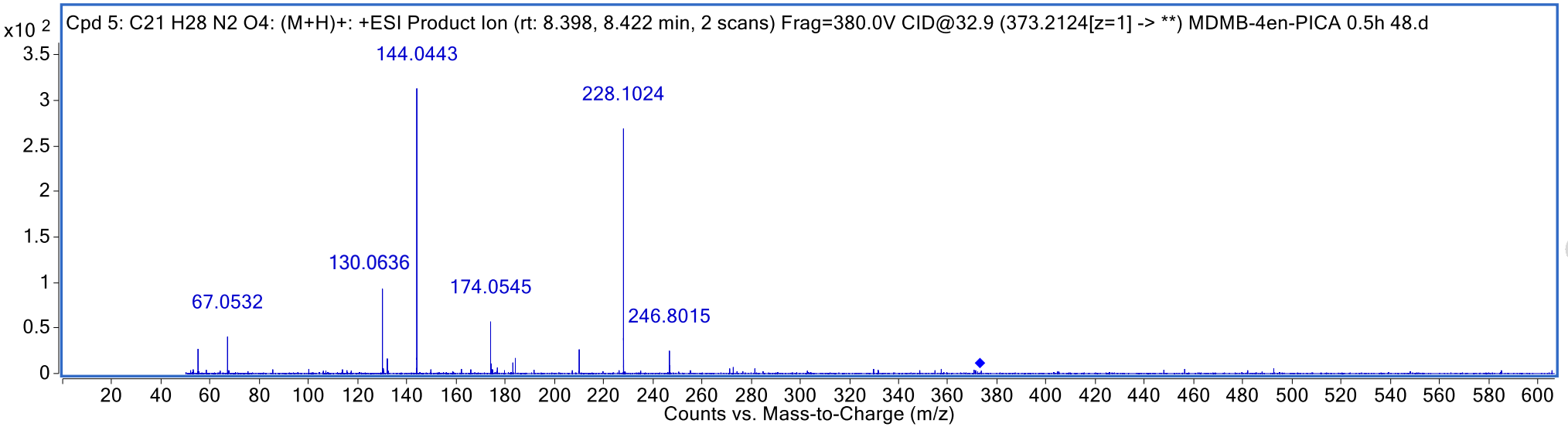
[M+H]+
373.2122

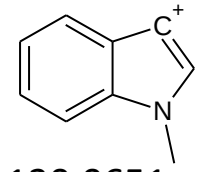


228.1019

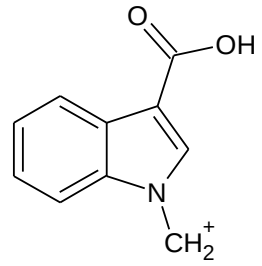


144.0444

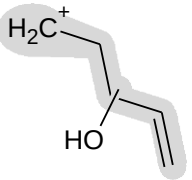




130.0651



174.0550



85.0648
(not detected)

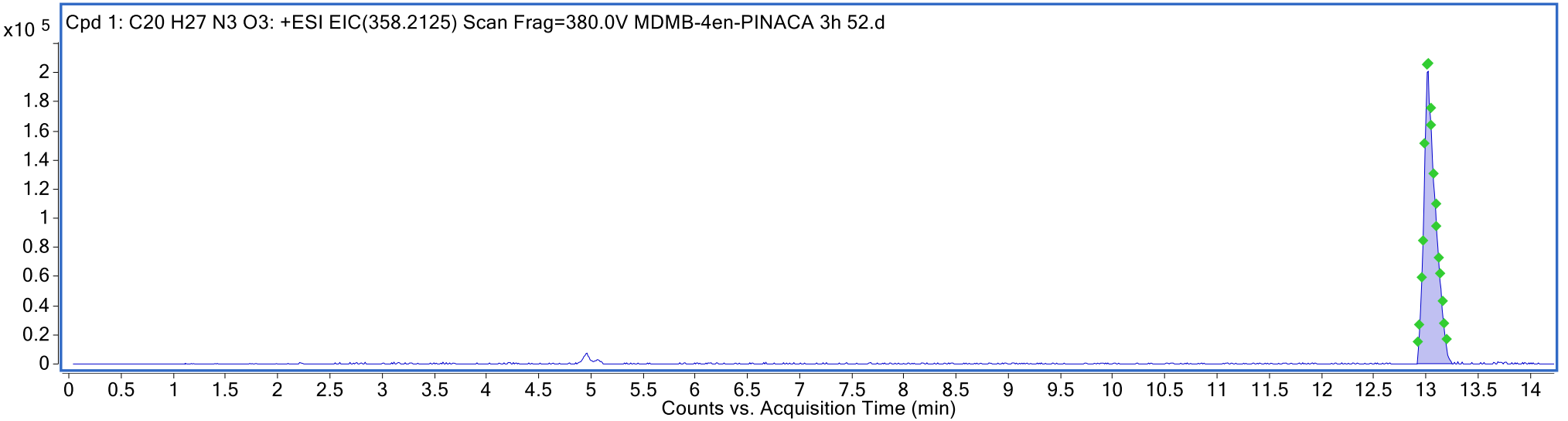
$-H_2O$

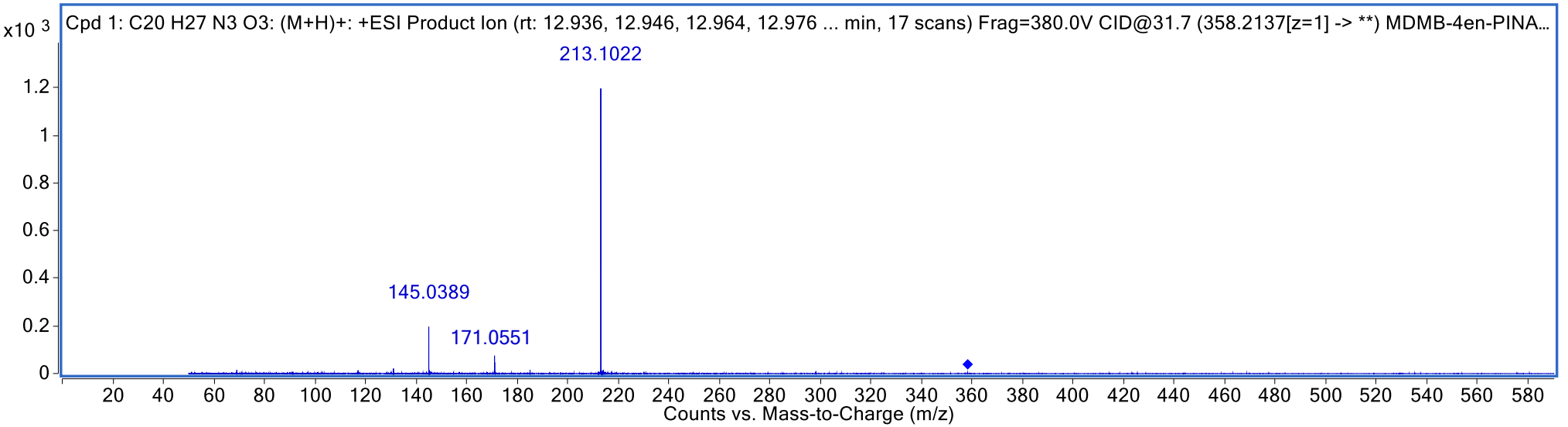
67.0542

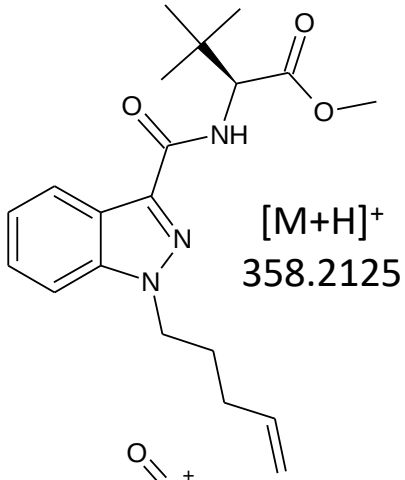
MDMB-4en-PINACA

Metabolism

MDMB-4en-PINACA, RT 13.02 min, *m/z* 358.2161







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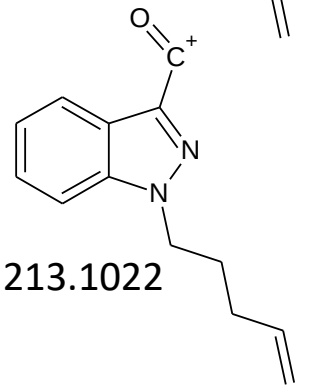
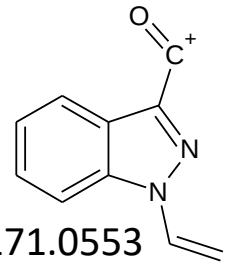


213.1022



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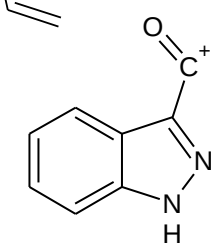
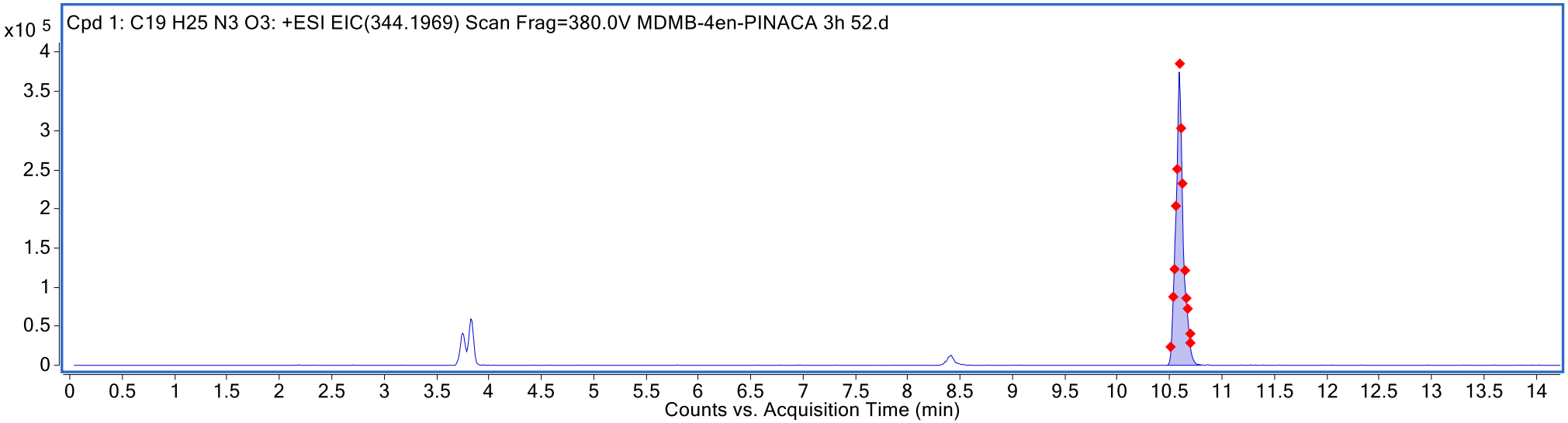
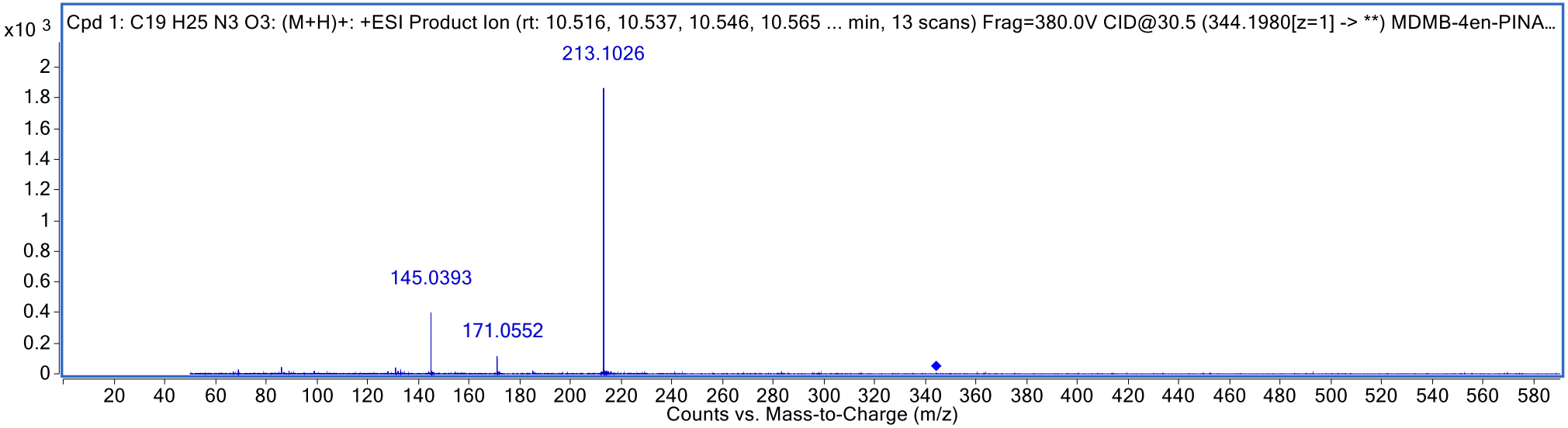
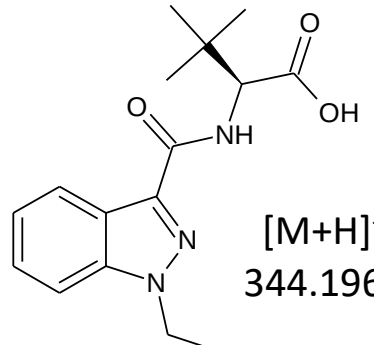


145.0396

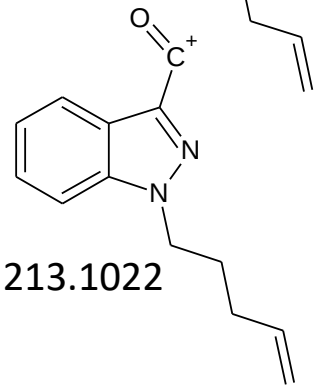
F1, Ester hydrolysis, RT 10.59 min, *m/z* 344.1981







$[M+H]^+$
344.1969



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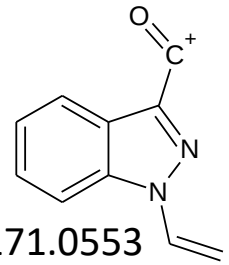

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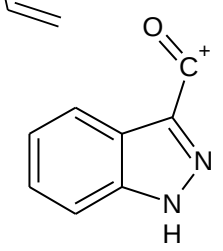
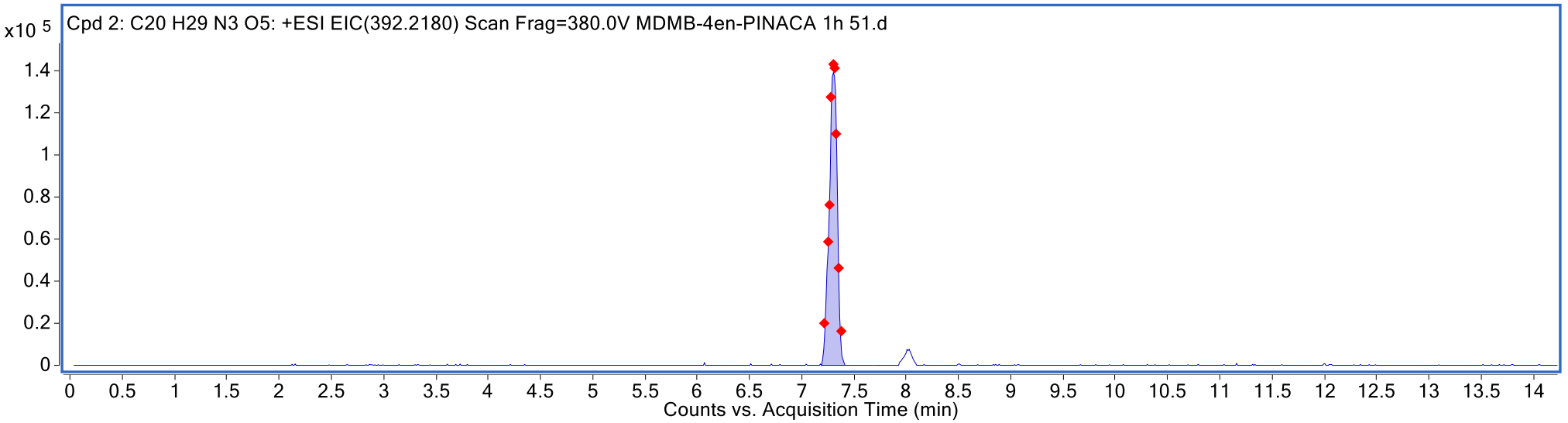
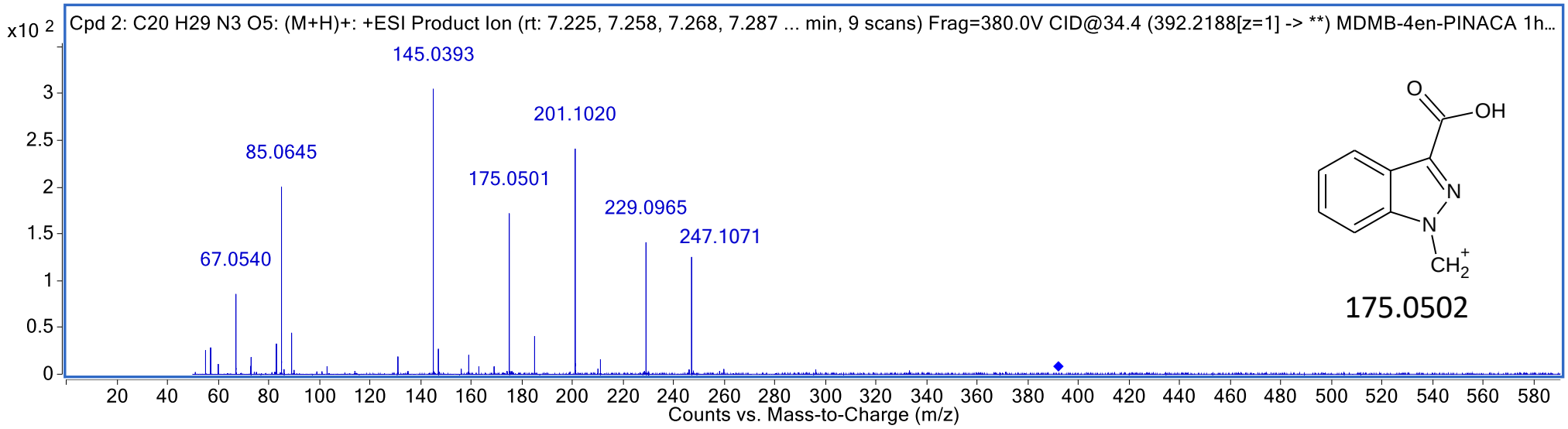
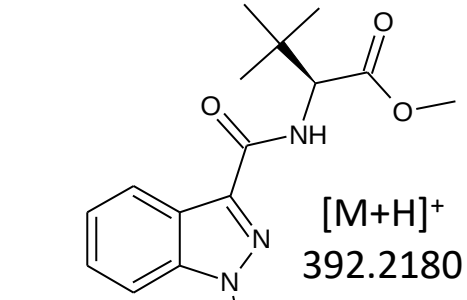


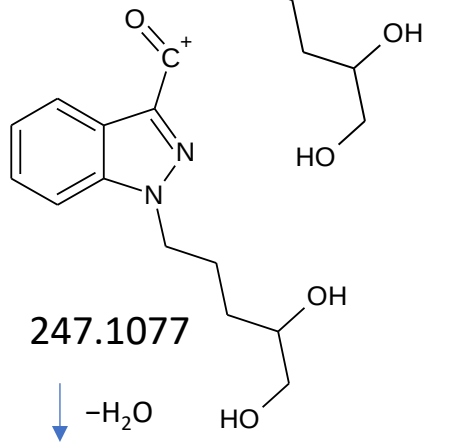
145.0396

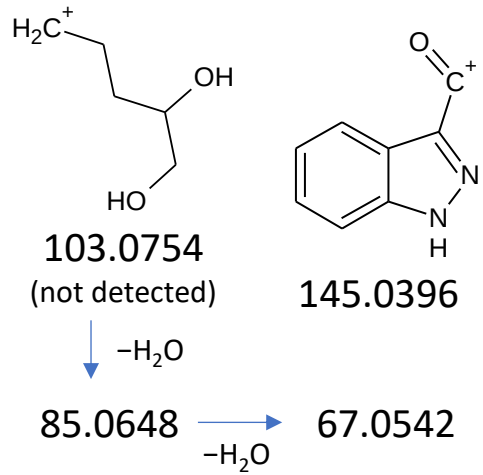
F2, Dihydrodiol formation, RT 7.29 min, *m/z* 392.2188



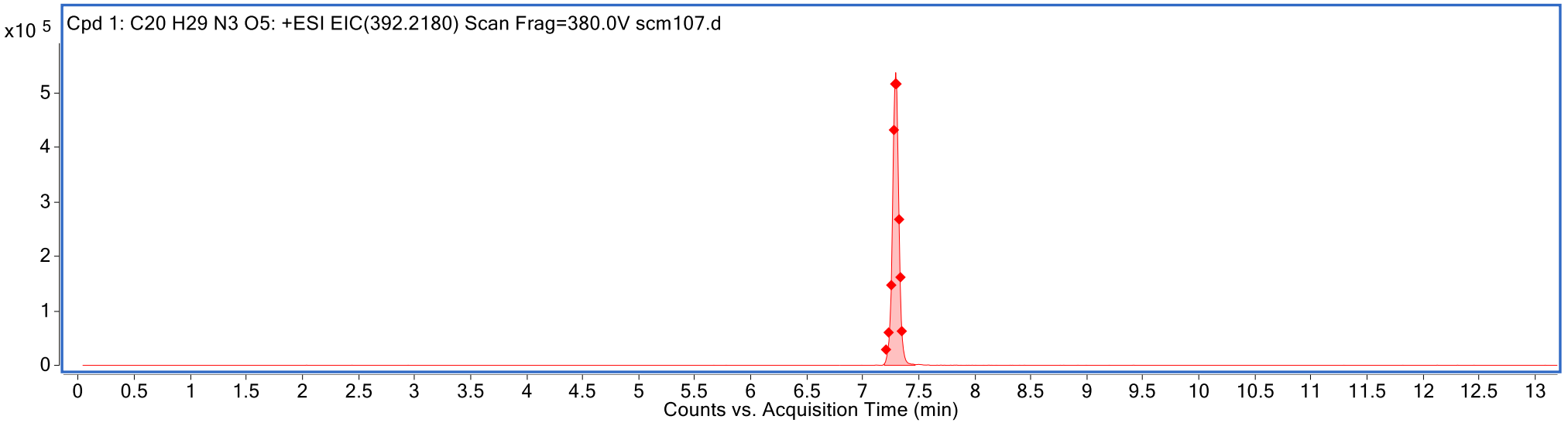


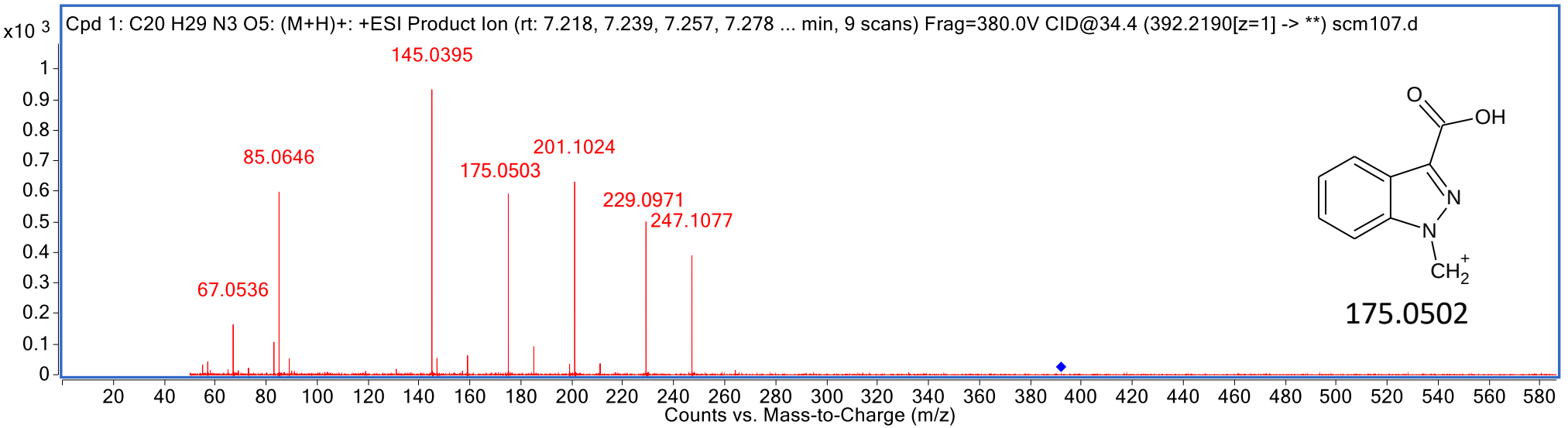


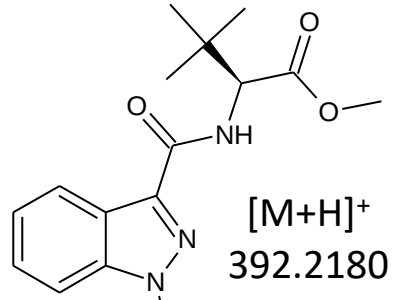


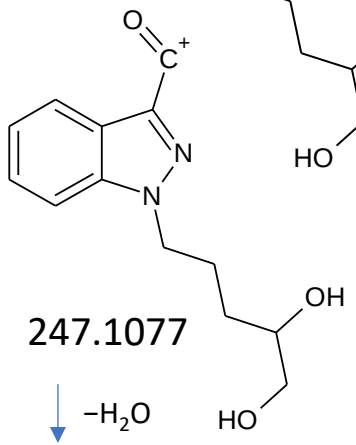


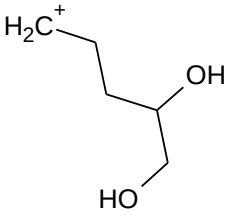
Dihydrodiol reference standard, RT 7.29 min, *m/z* 392.2190

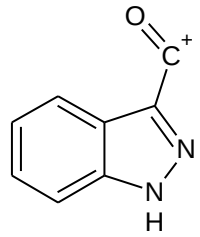




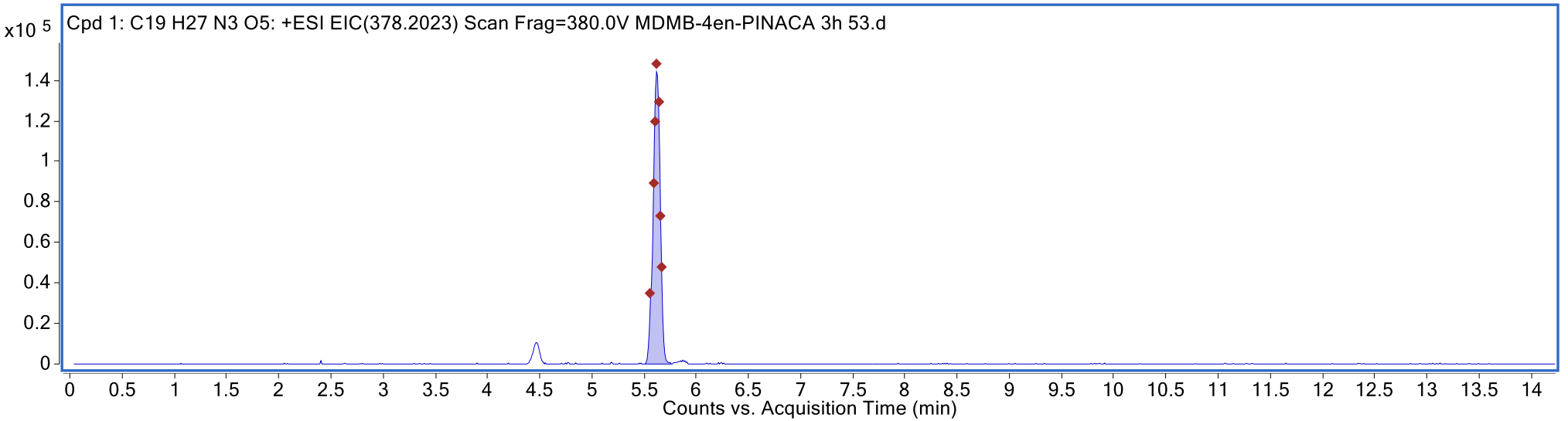


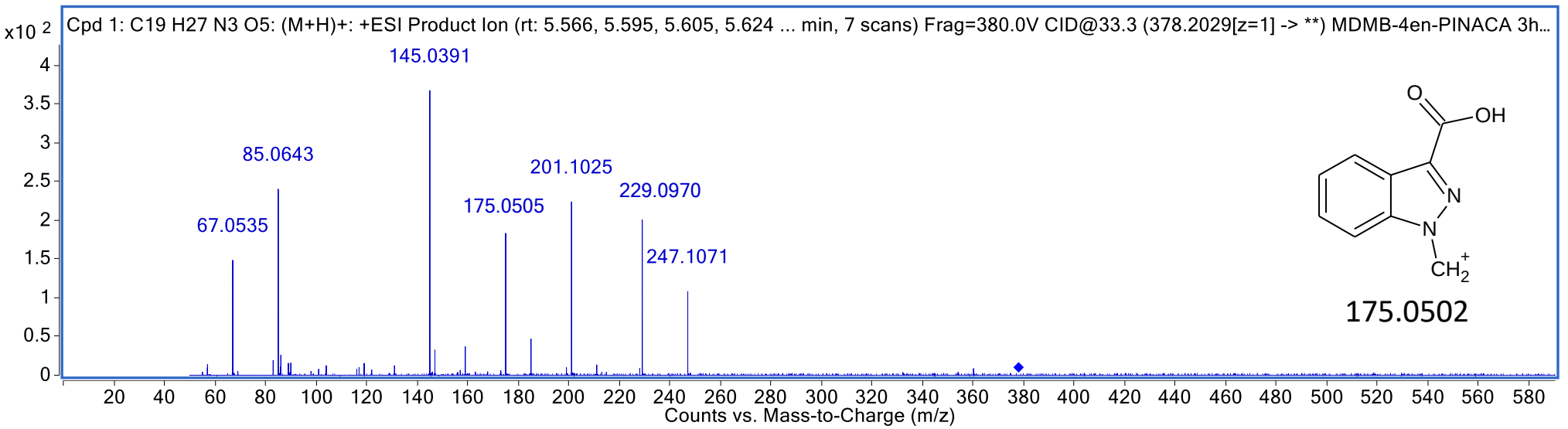


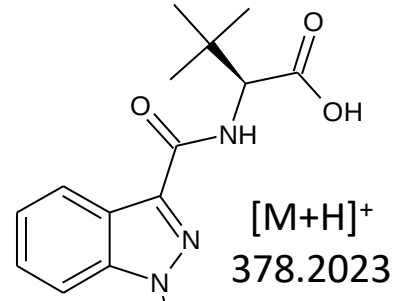


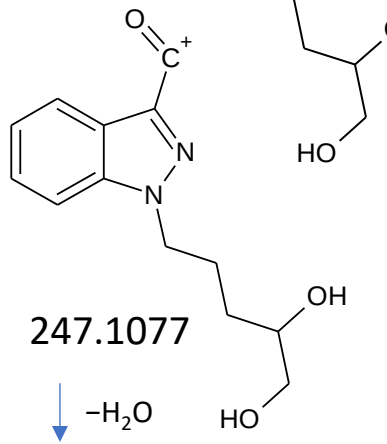


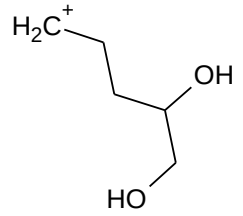
F3, Ester hydrolysis + dihydrodiol formation, RT 5.62 min, *m/z* 378.2026

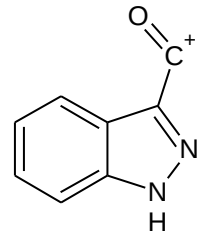




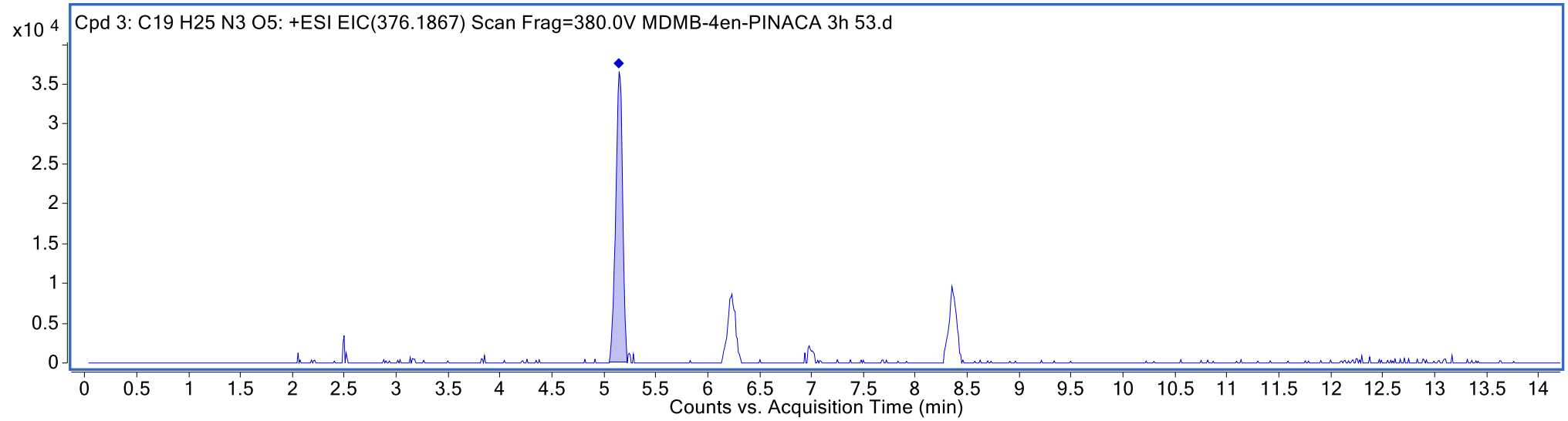


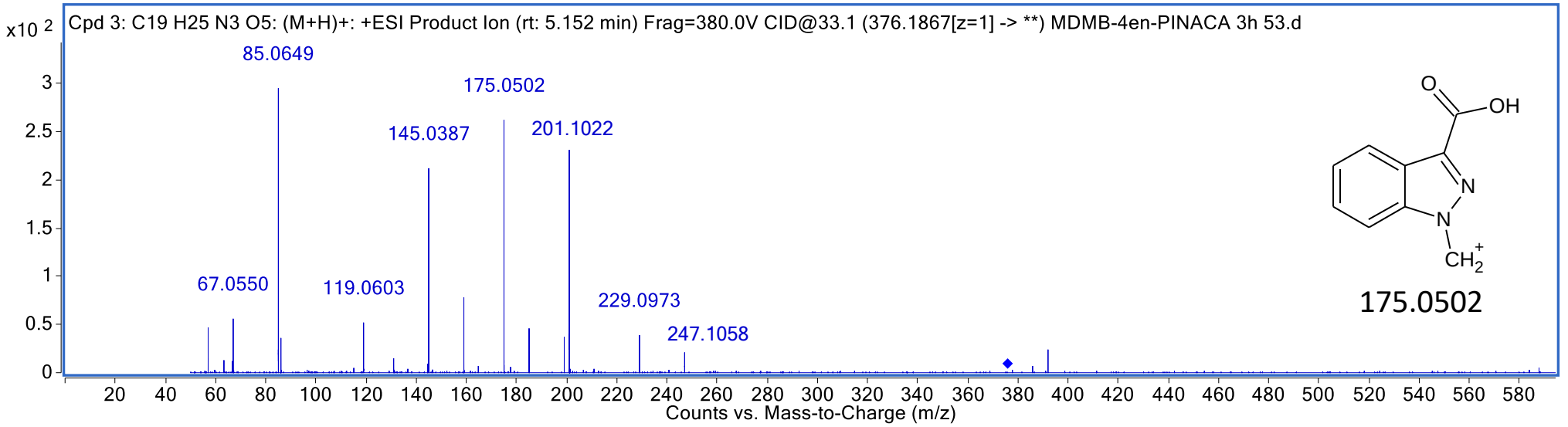


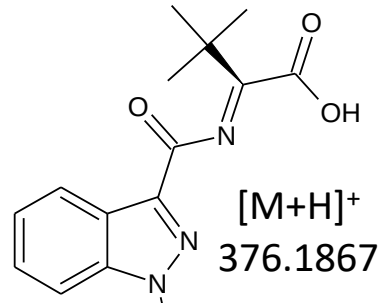


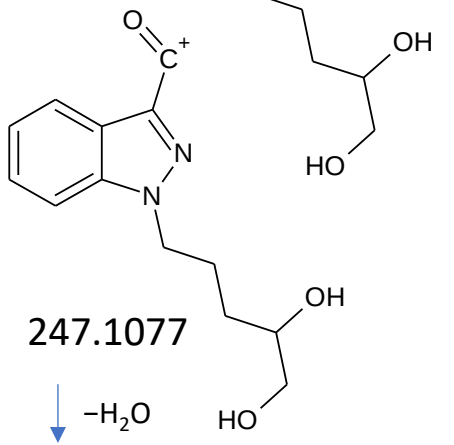


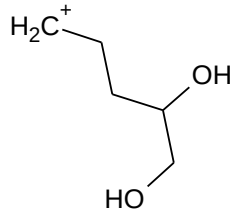
F4, Ester hydrolysis + dihydrodiol formation + dehydrogenation, RT 5.14 min, *m/z* 376.1870

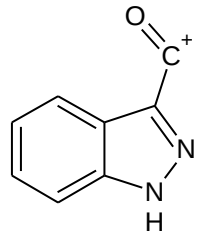




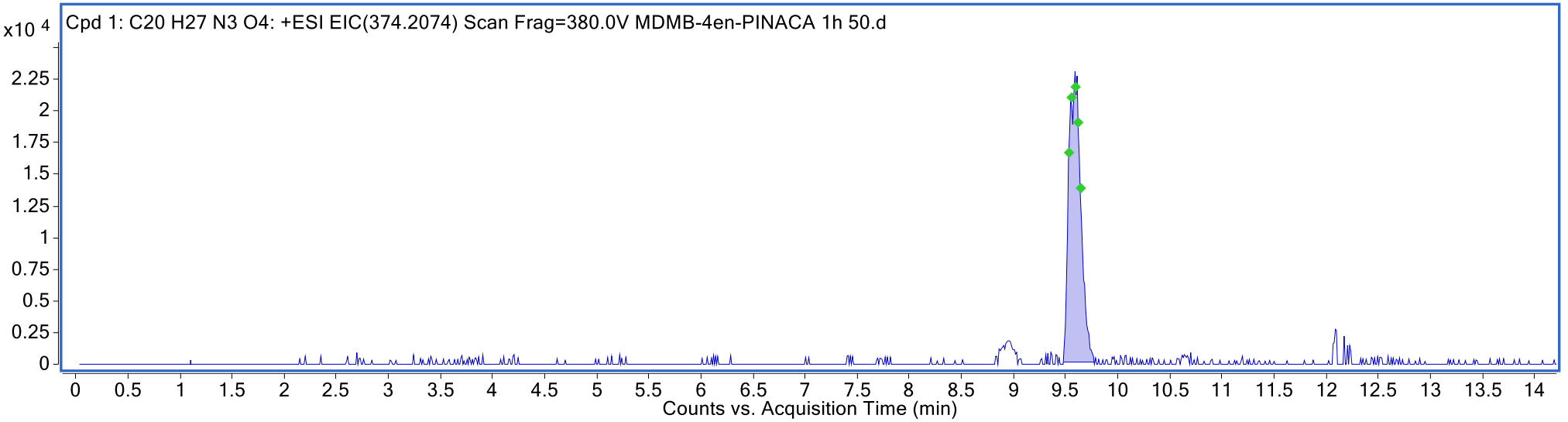


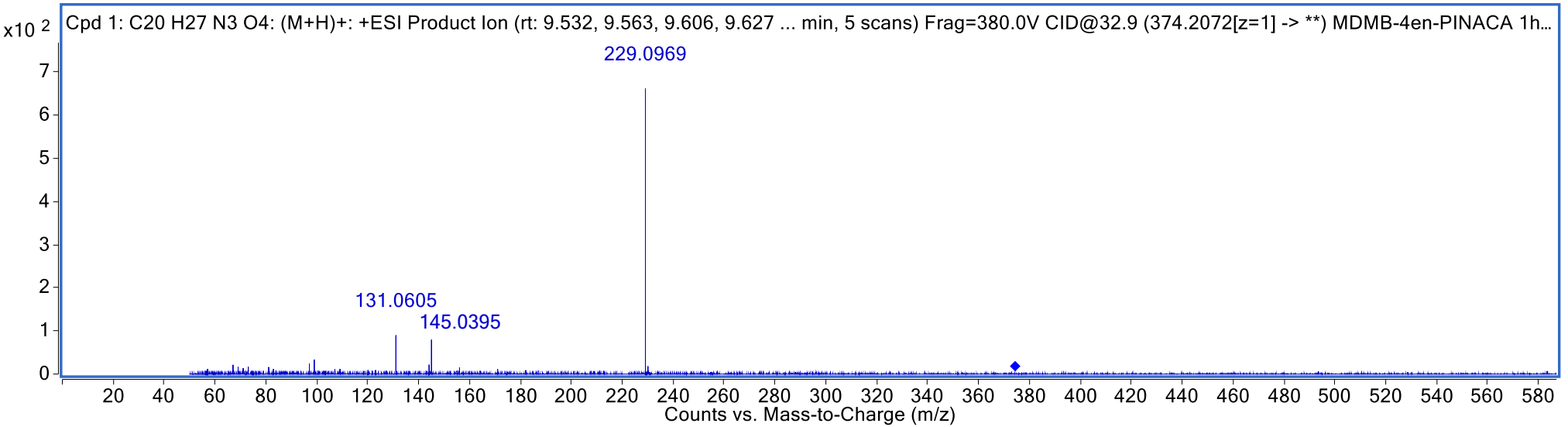


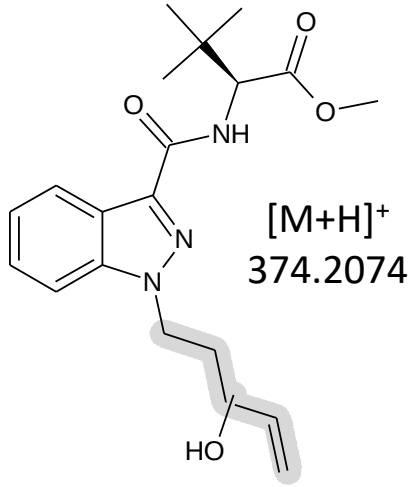




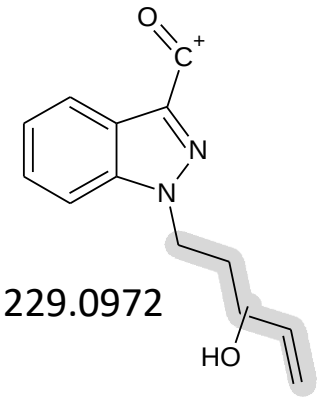
F5, Mono-hydroxylation (pentenyl tail), RT 9.59 min, *m/z* 374.2077



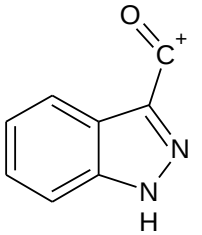




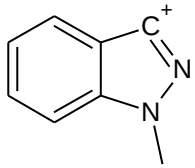
$[M+H]^+$
374.2074



229.0972

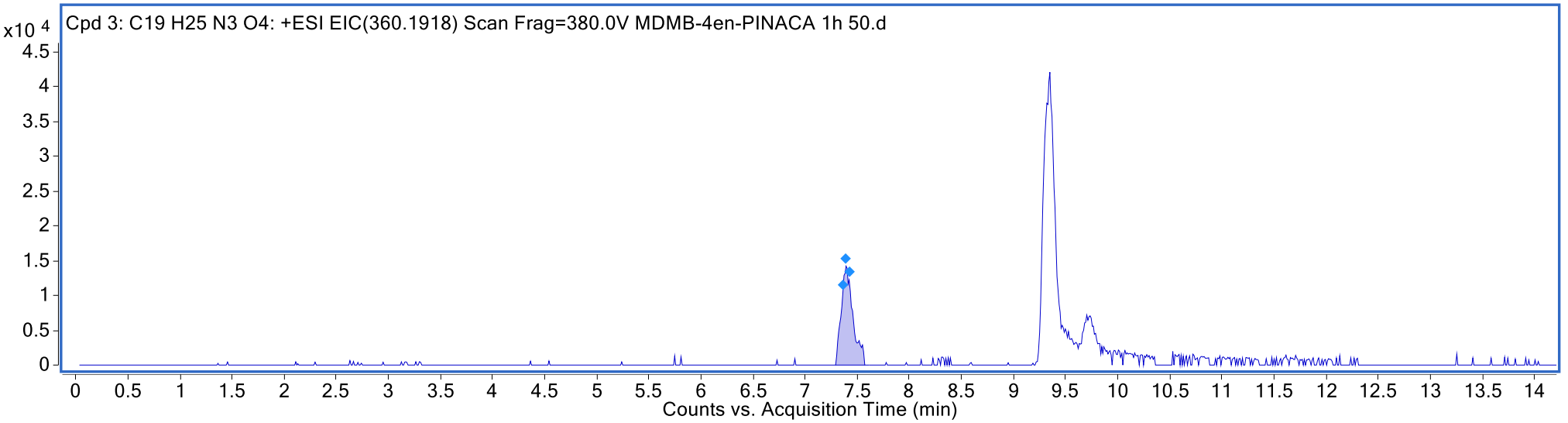


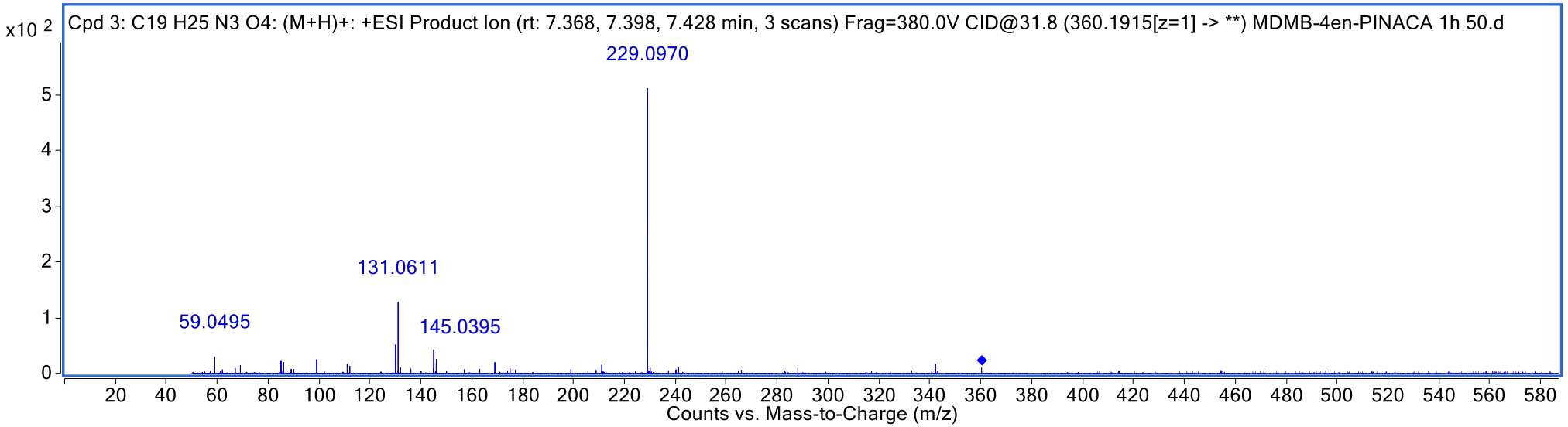
145.0396

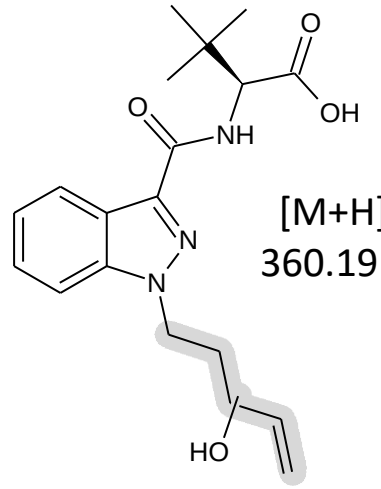


131.0604

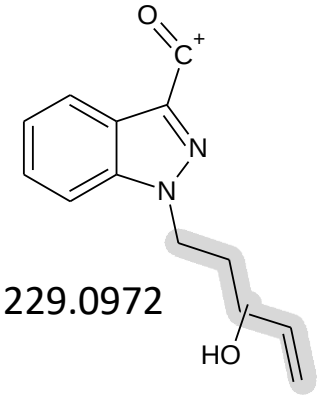
F6, Ester hydrolysis + mono-hydroxylation (pentenyl tail), RT 7.39 min, *m/z* 360.1913



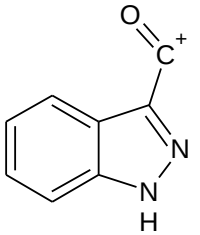




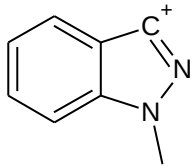
$[M+H]^+$
360.1918



229.0972

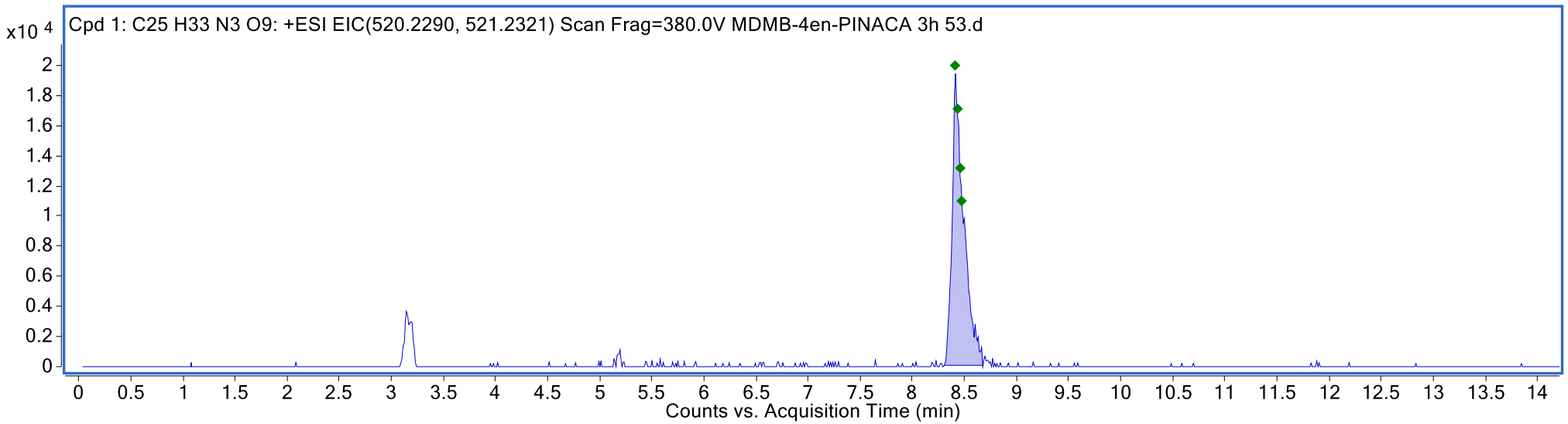


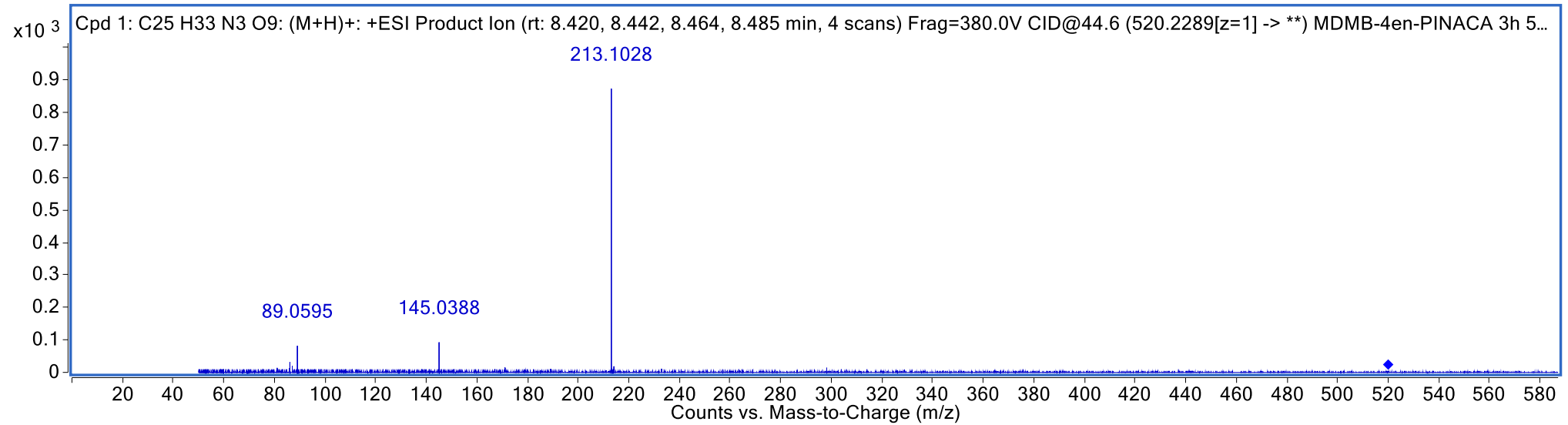
145.0396

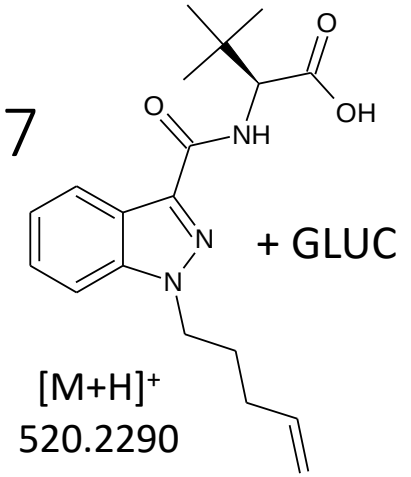


131.0604

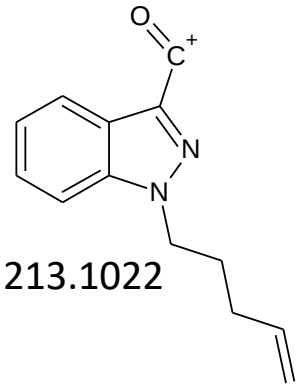
F7, Ester hydrolysis + glucuronidation, RT 8.41 min, *m/z* 520.2287



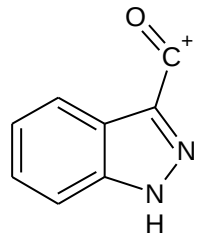




[M+H]⁺
520.2290

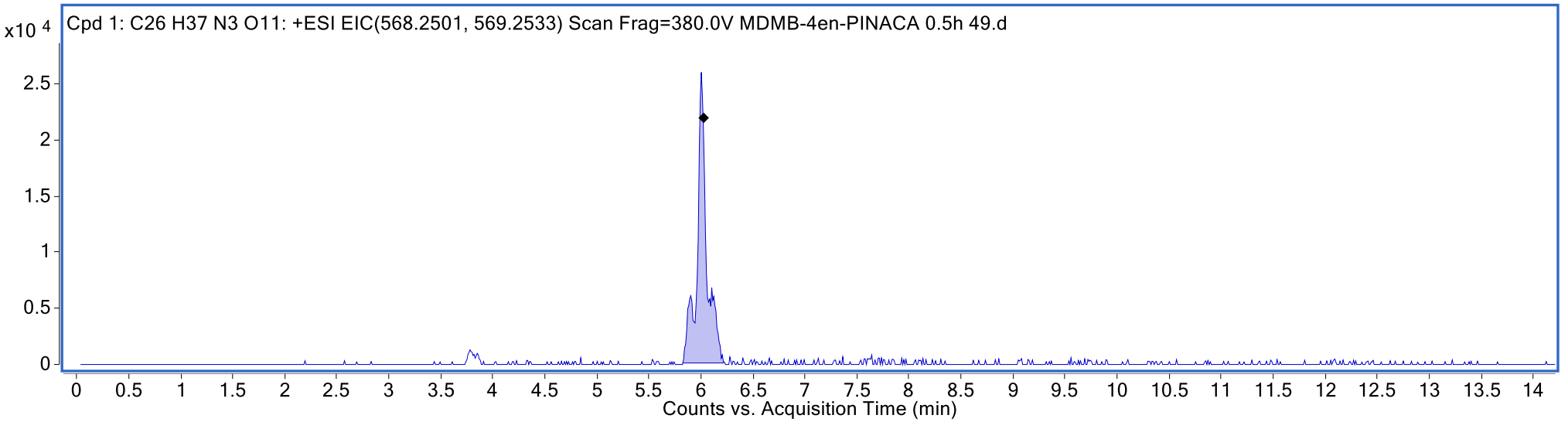


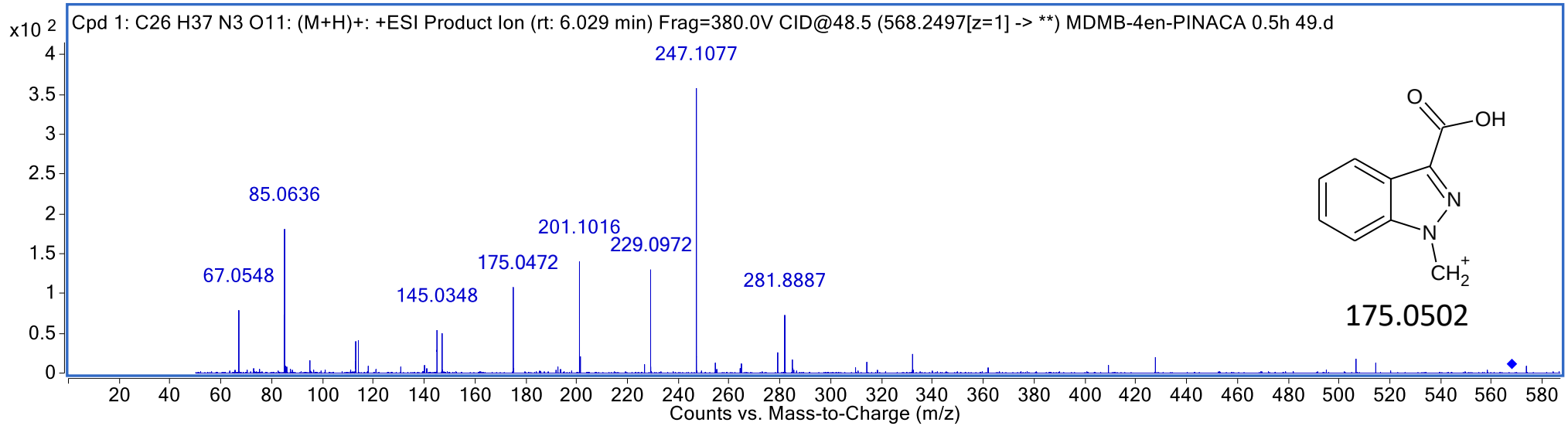
213.1022

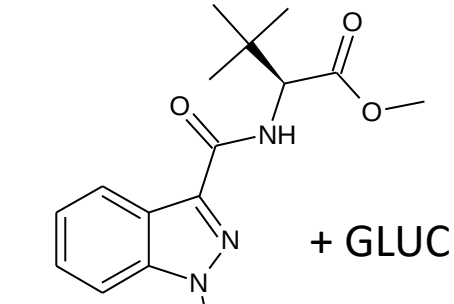


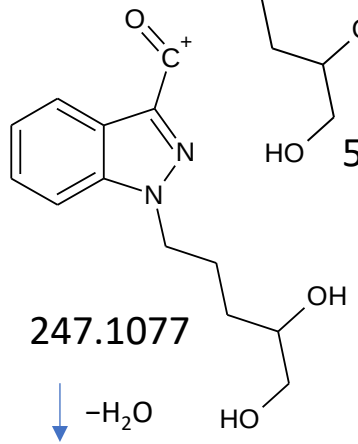
145.0396

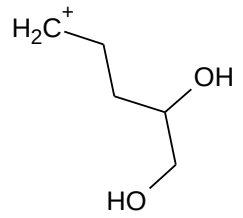
F8, Dihydrodiol formation + glucuronidation, RT 6.02 min, *m/z* 568.2497

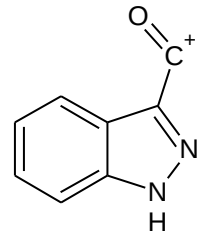




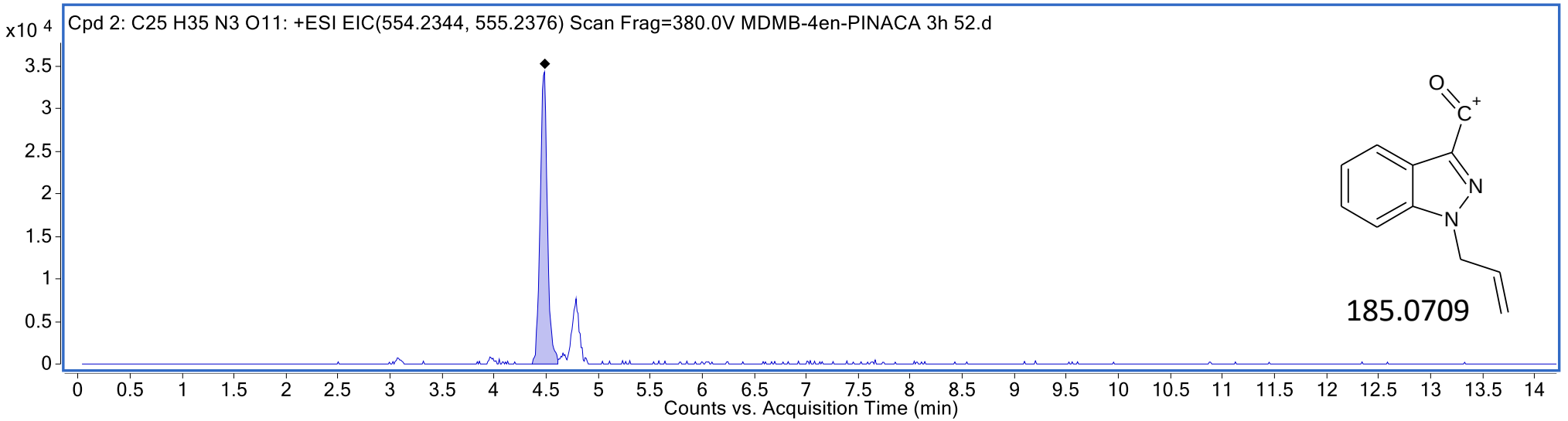


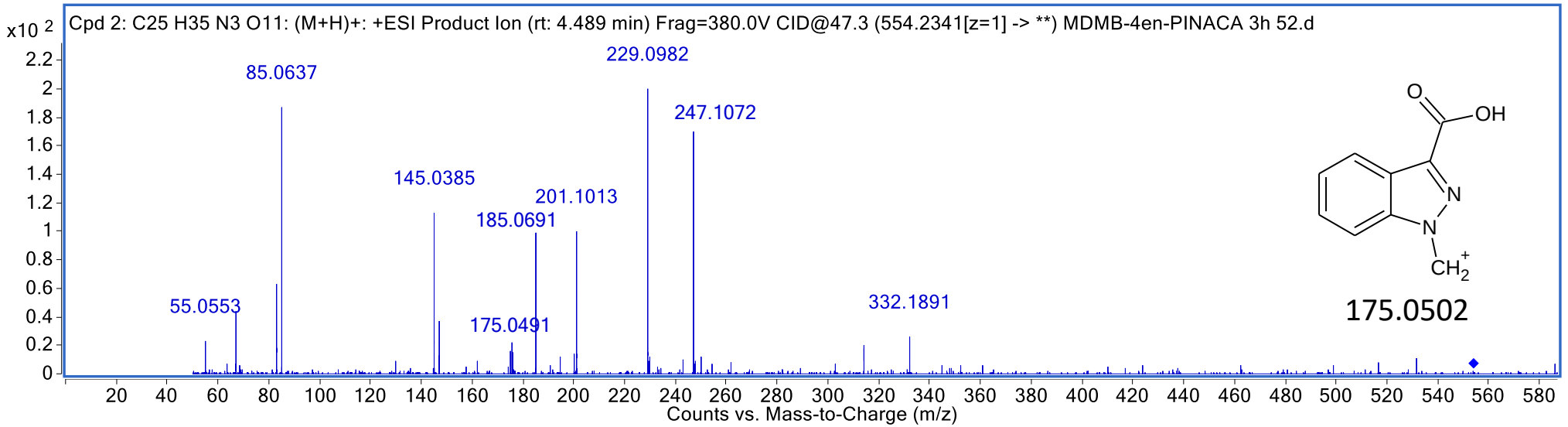


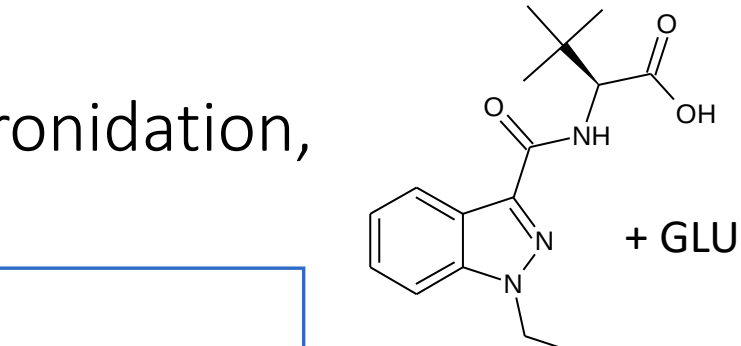


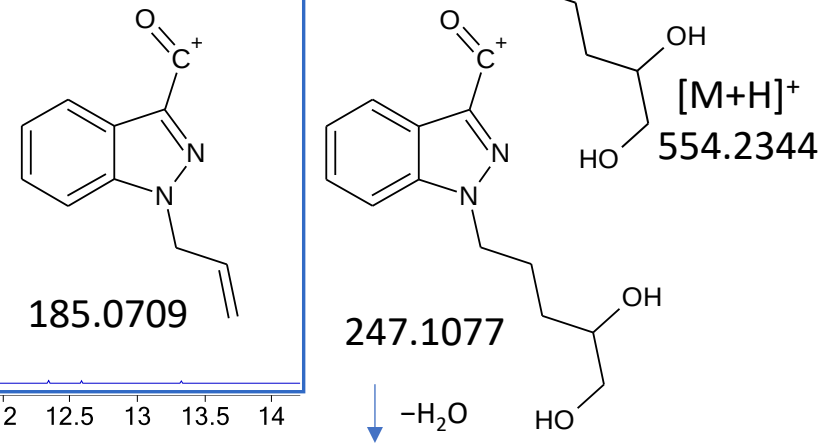


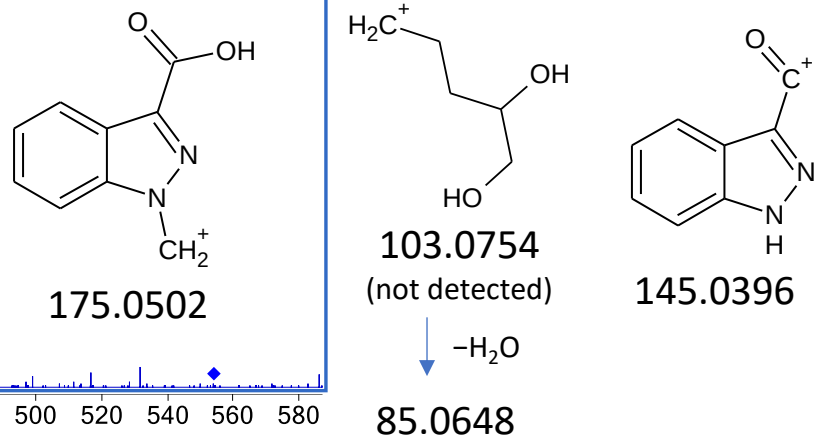
F9, Ester hydrolysis + dihydrodiol formation + glucuronidation, RT 4.47 min, *m/z* 554.2337







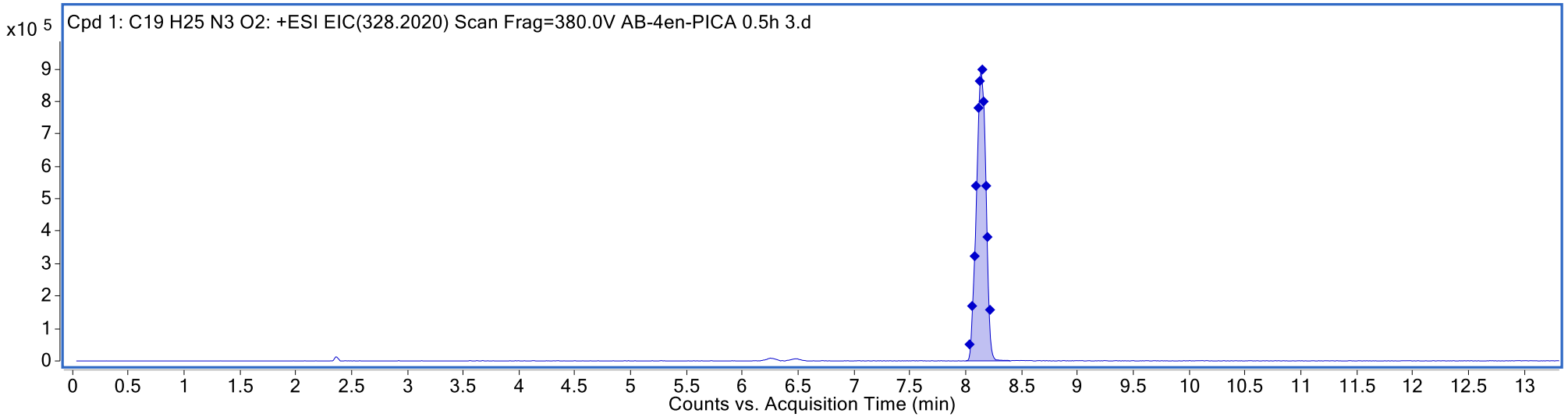


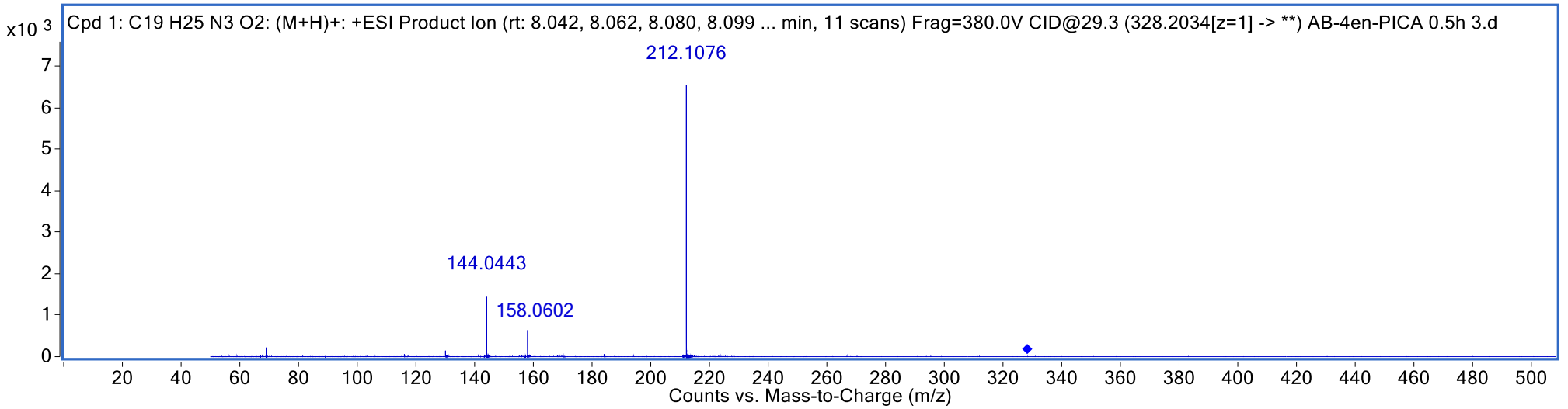


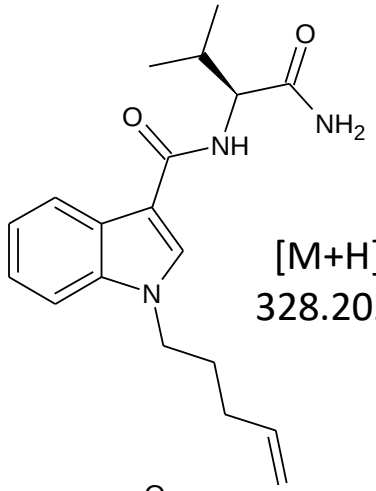
AB-4en-PICA

Metabolism

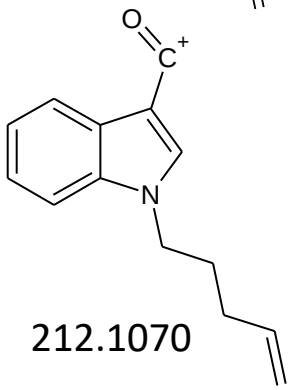
AB-4en-PICA, RT 8.14 min, *m/z* 328.2034



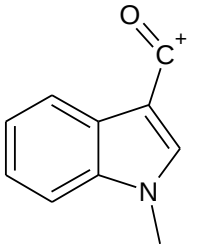




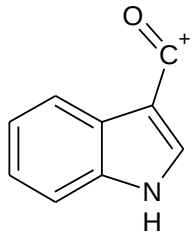
[M+H]+
328.2020



212.1070

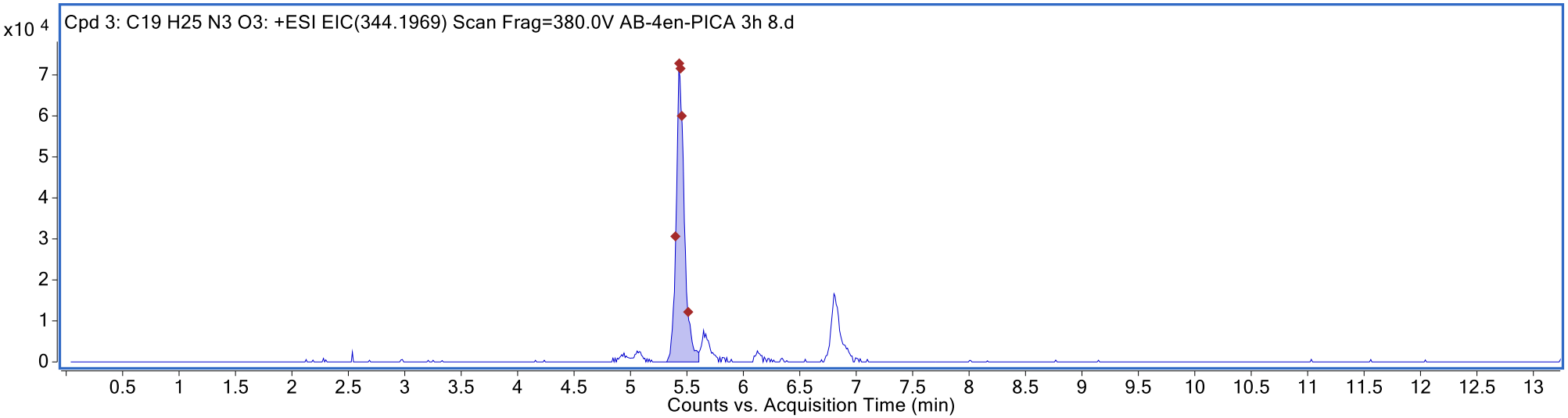


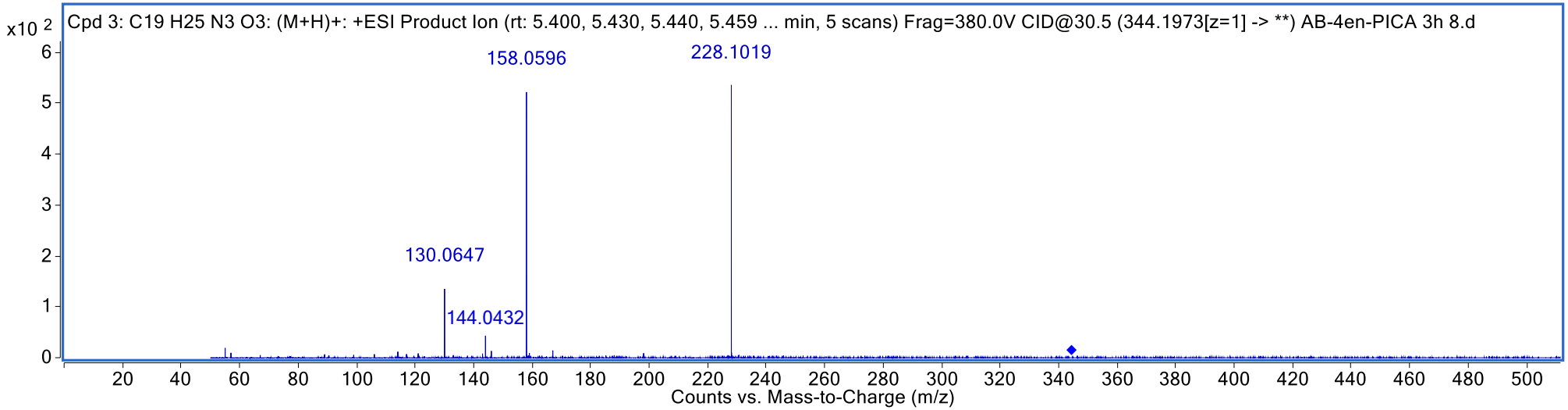
158.0600

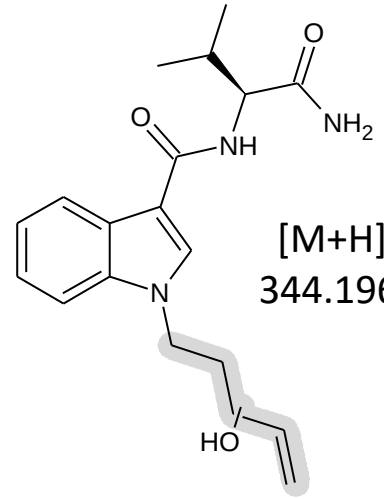


144.0444

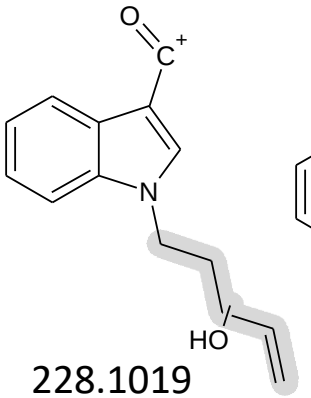
G1, Mono-hydroxylation (pentenyl tail), RT 5.45 min, *m/z* 344.1973



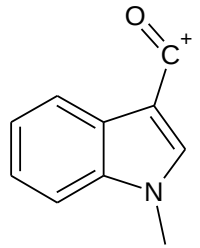




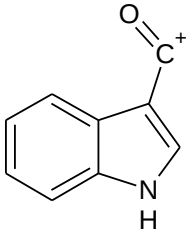
[M+H]+
344.1969



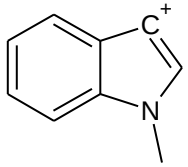
228.1019



158.0600

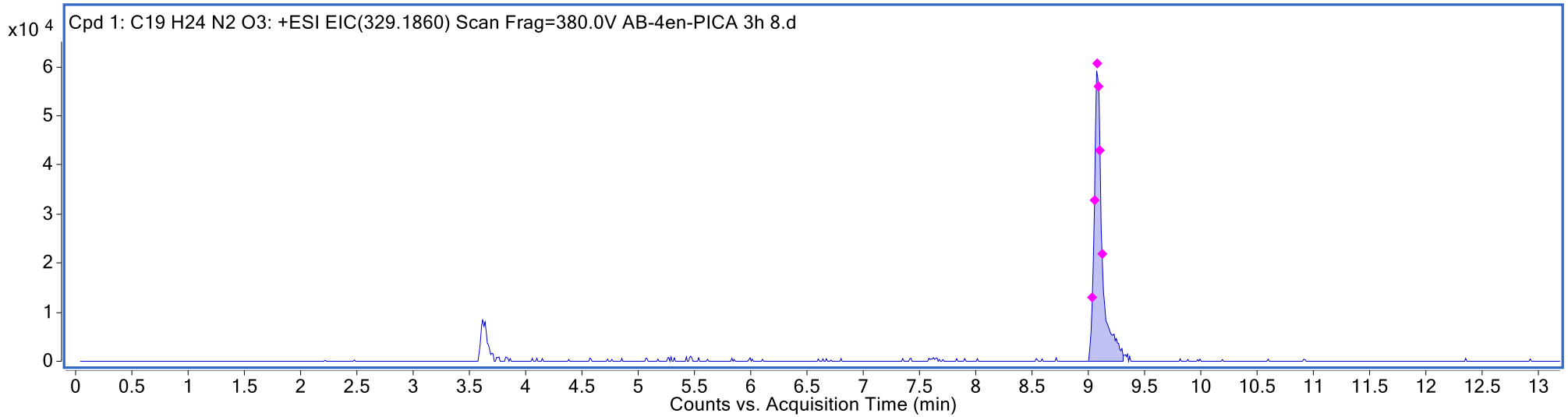


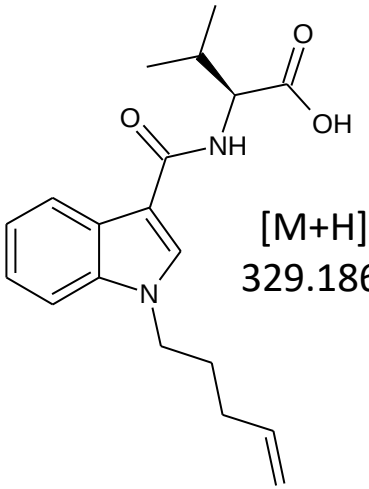
144.0444



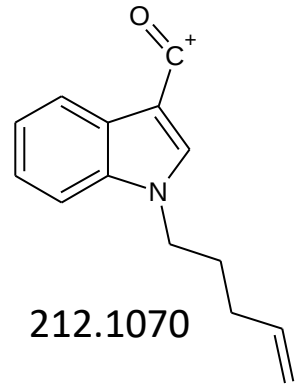
130.0651

G2, Terminal amide hydrolysis, RT 9.08 min, *m/z* 329.1862

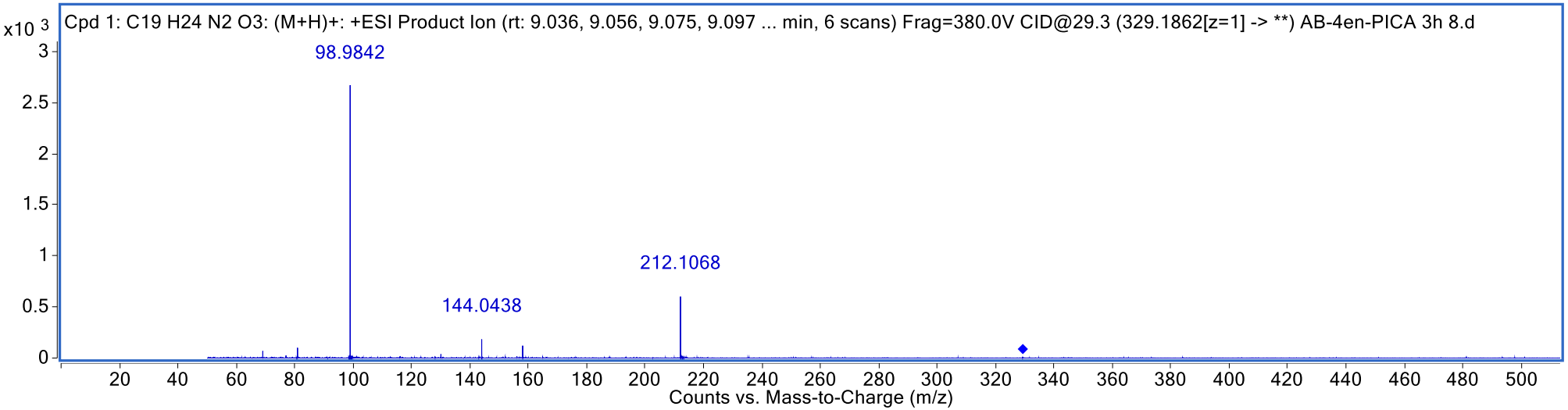


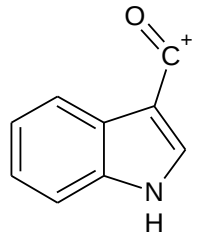


$[M+H]^+$
329.1860



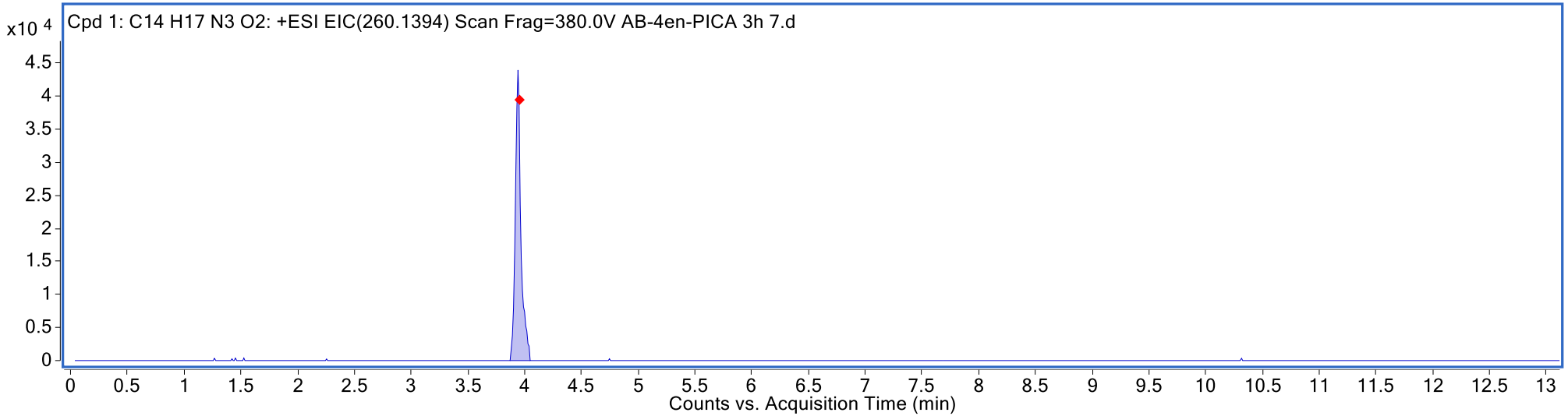
212.1070

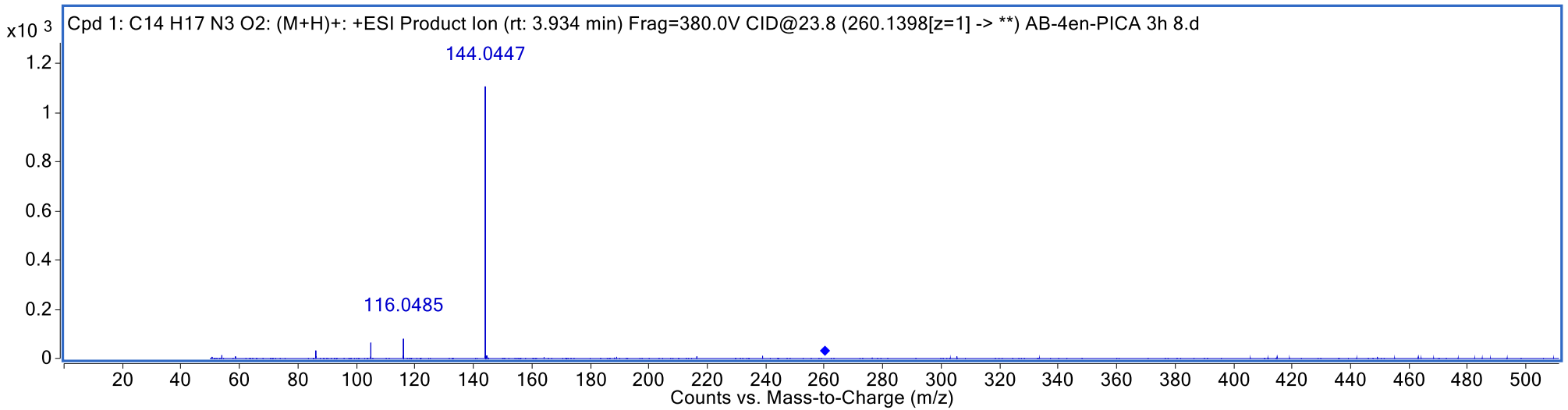


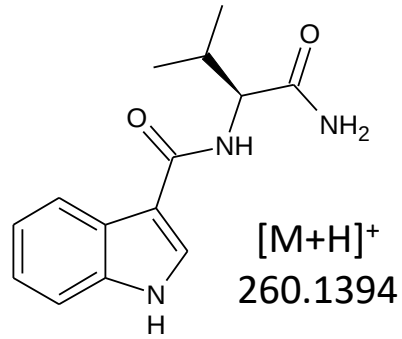


144.0444

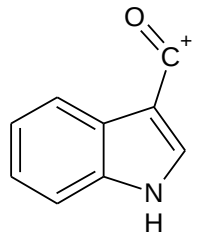
G3, *N*-dealkylation, RT 3.95 min, *m/z* 260.1395



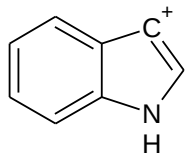




[M+H]+
260.1394

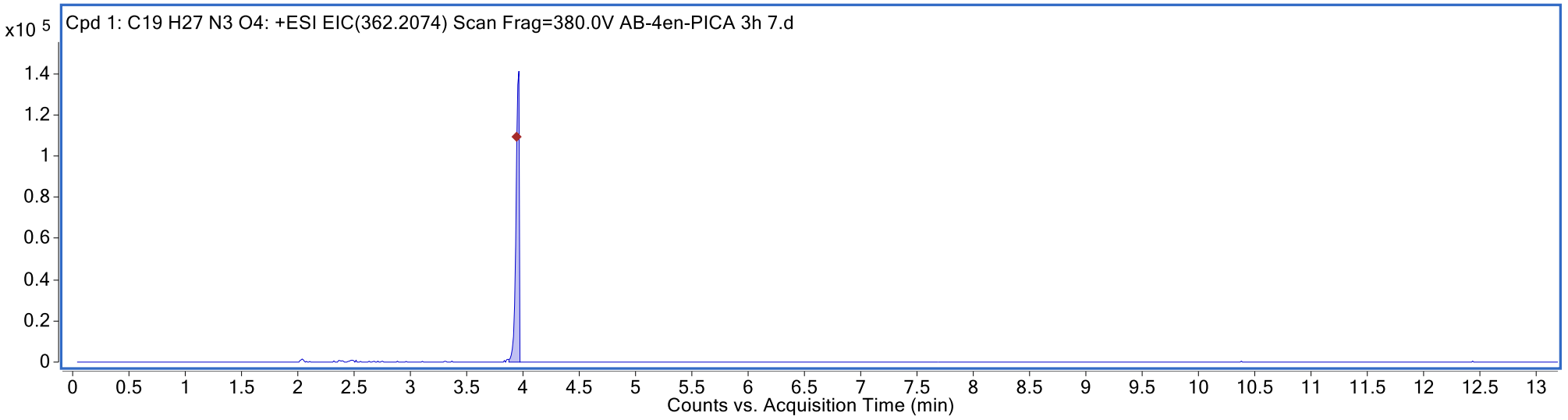


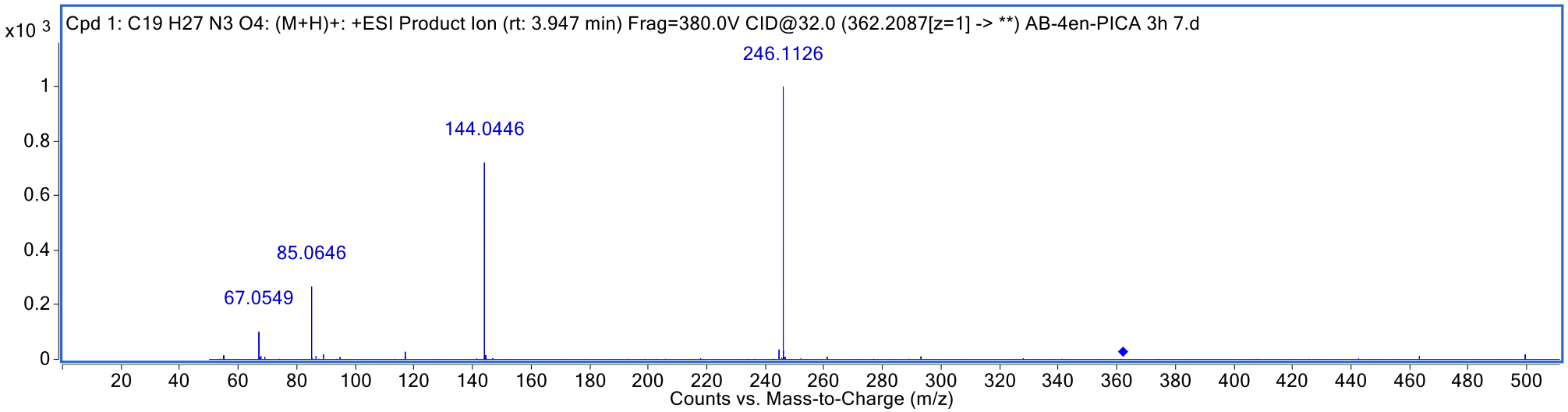
144.0444

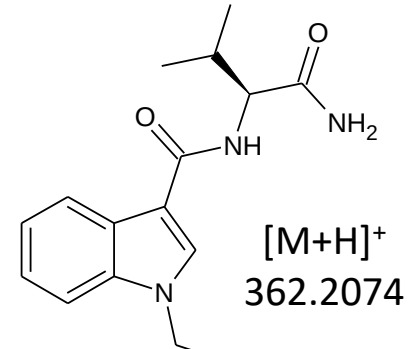


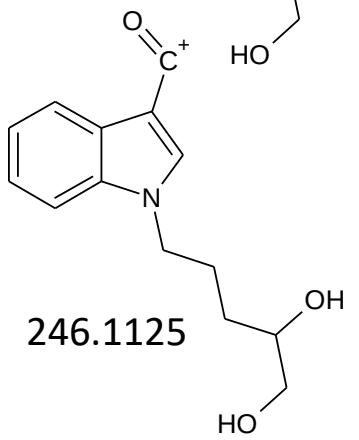
116.0495

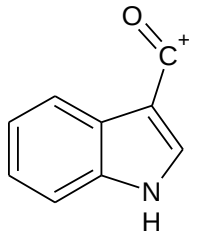
G4, Dihydrodiol formation, RT 3.97 min, *m/z* 362.2076

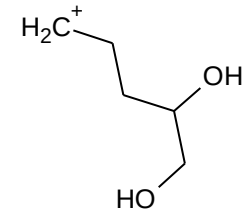




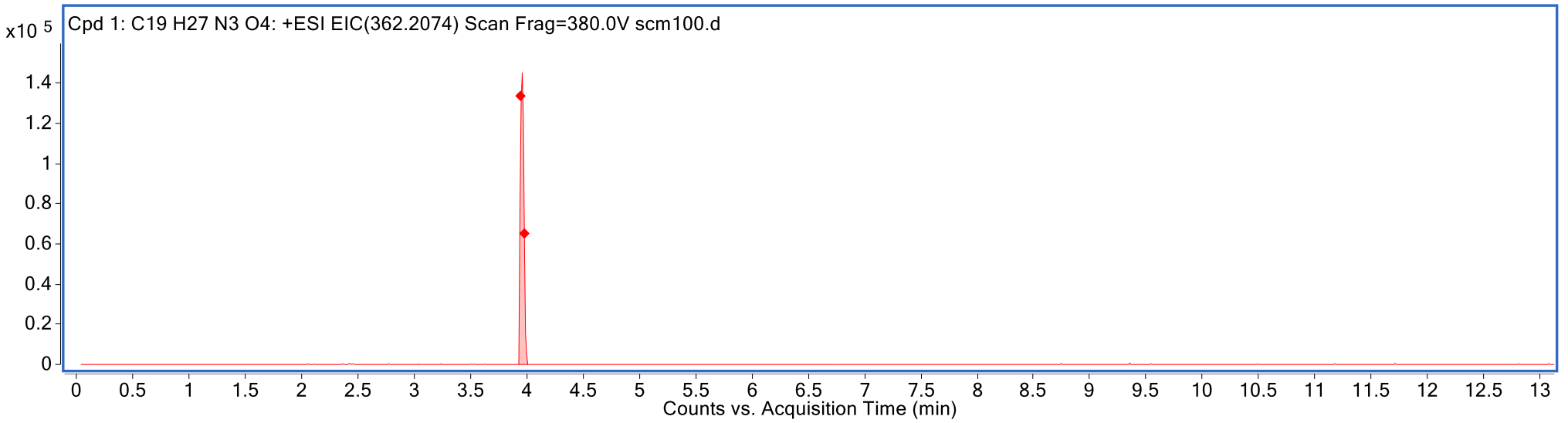


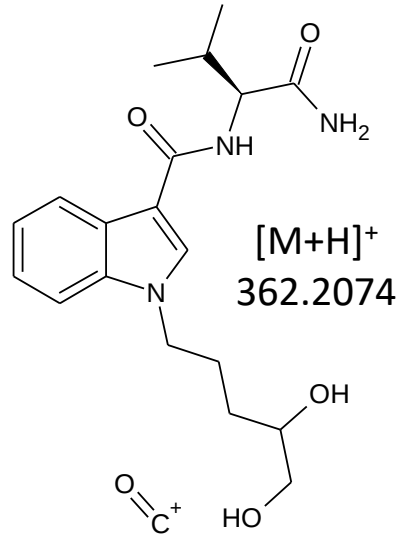




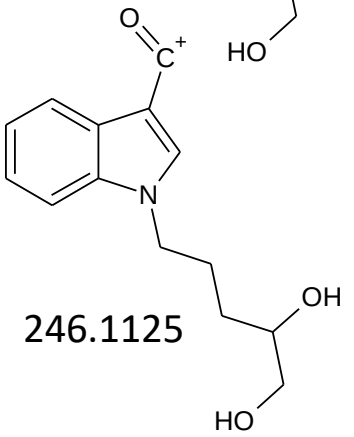


Dihydrodiol reference standard, RT 3.96 min, *m/z* 362.2069

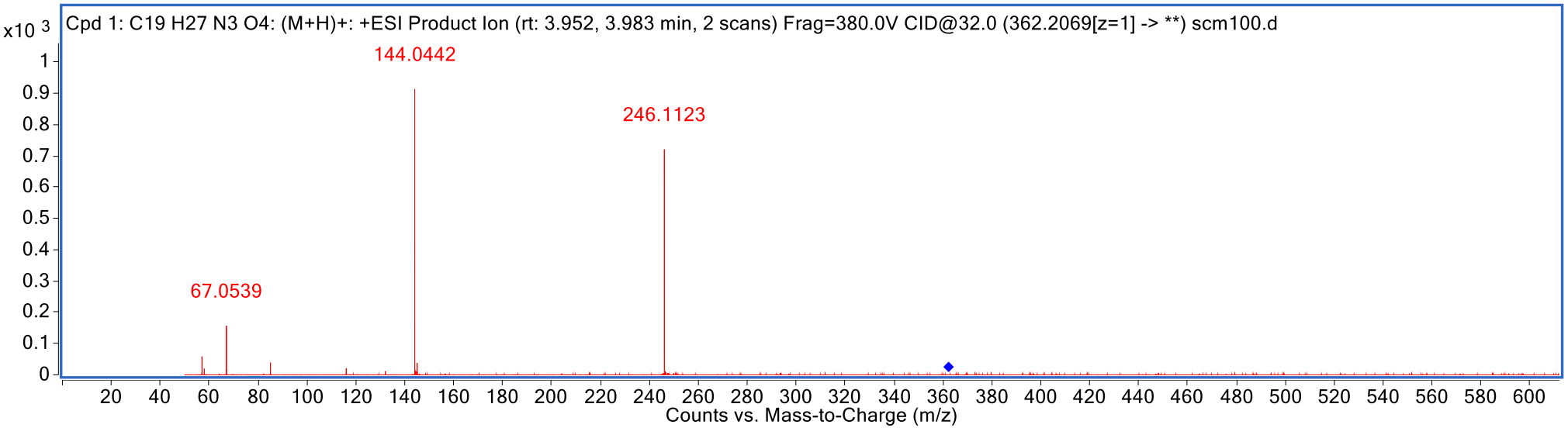


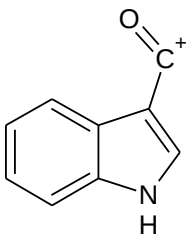


$[M+H]^+$
362.2074

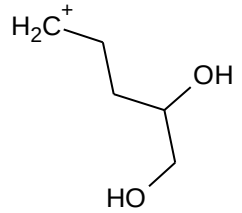


246.1125





144.0444



103.0754
(not detected)

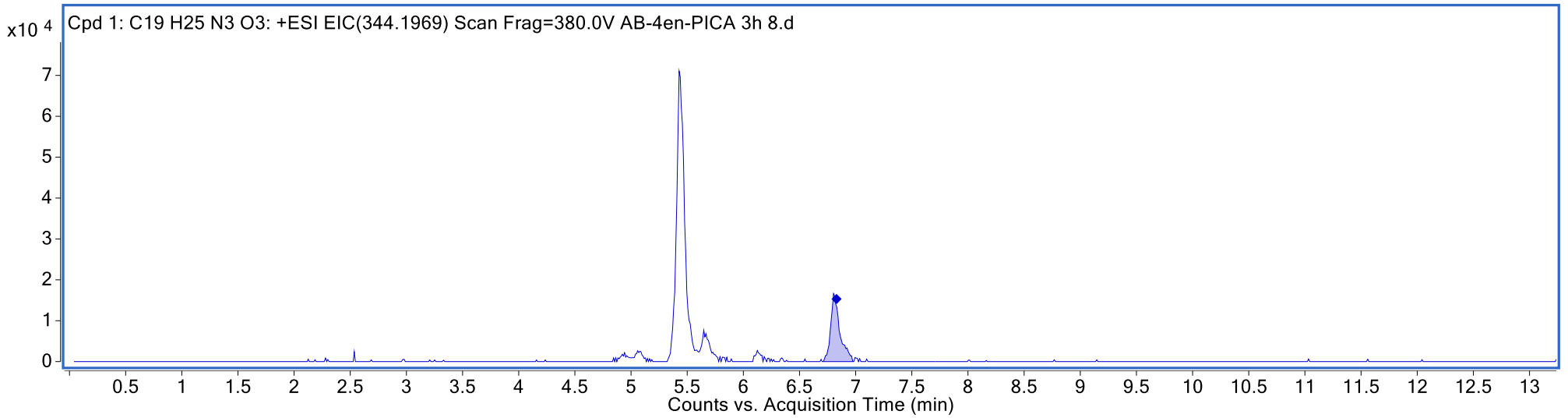
$-H_2O$

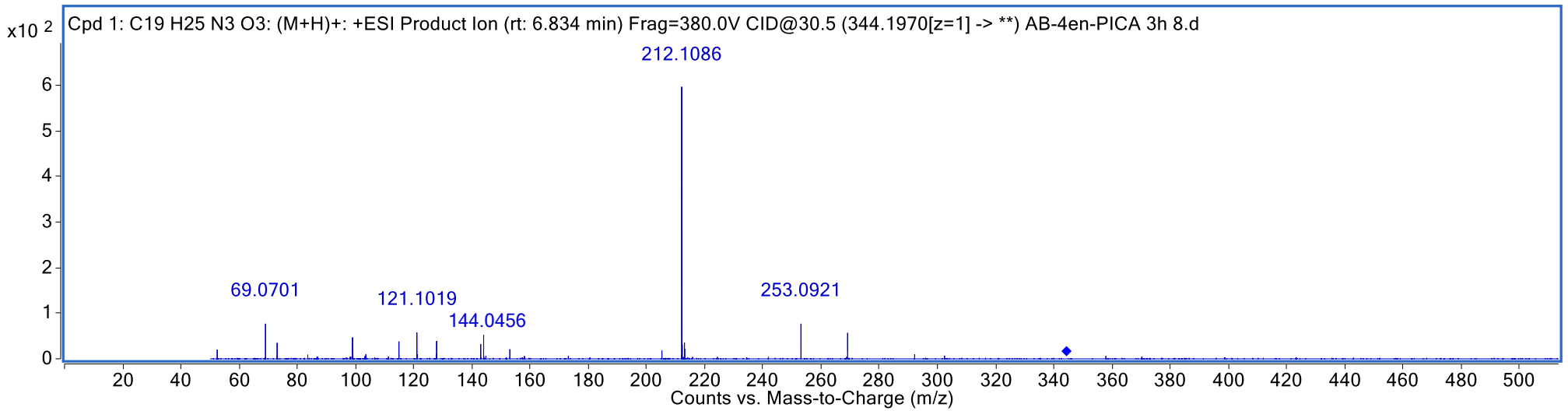
85.0648
(not detected)

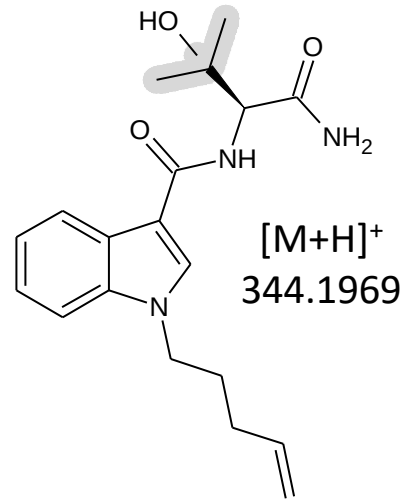
$-H_2O$

67.0542

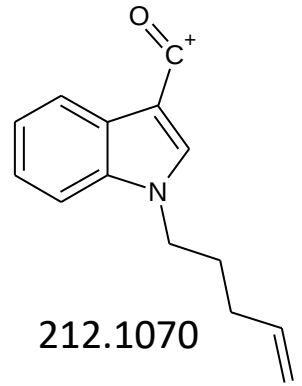
G5, Mono-hydroxylation (*iso*-propyl), RT 6.82 min, *m/z* 344.1970



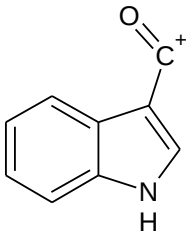




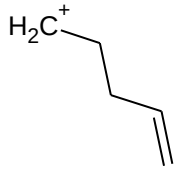
$[M+H]^+$
344.1969



212.1070



144.0444

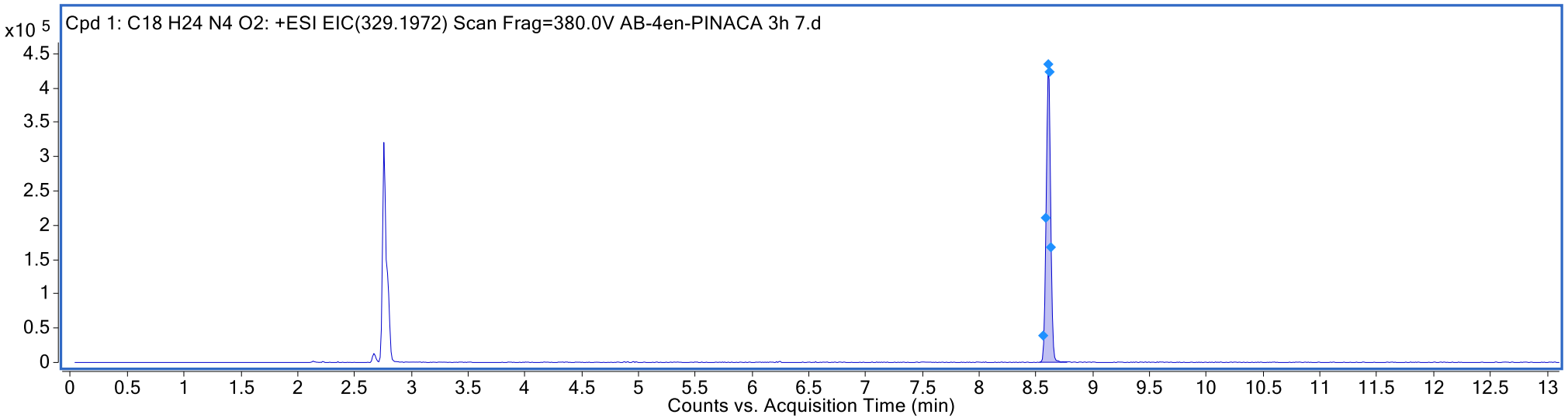


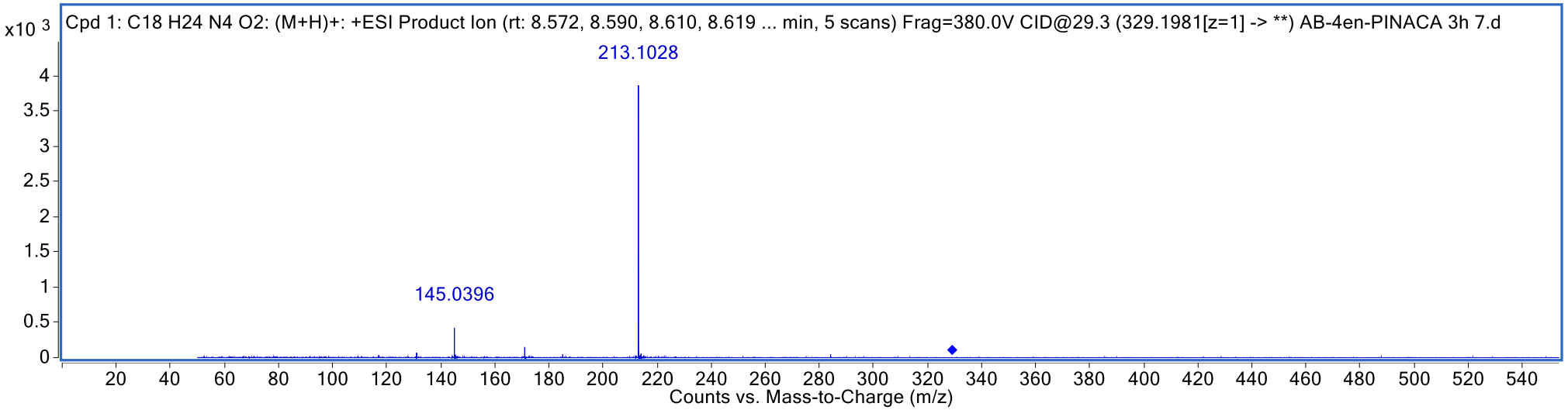
69.0699

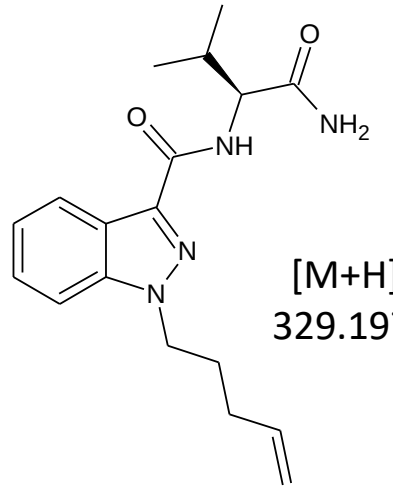
AB-4en-PINACA

Metabolism

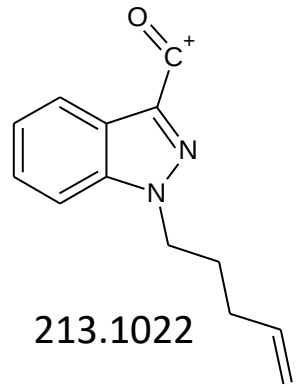
AB-4en-PINACA, RT 8.61 min, *m/z* 329.2005



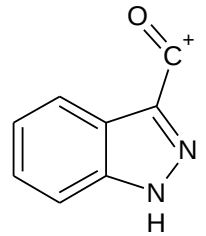




$[M+H]^+$
329.1972

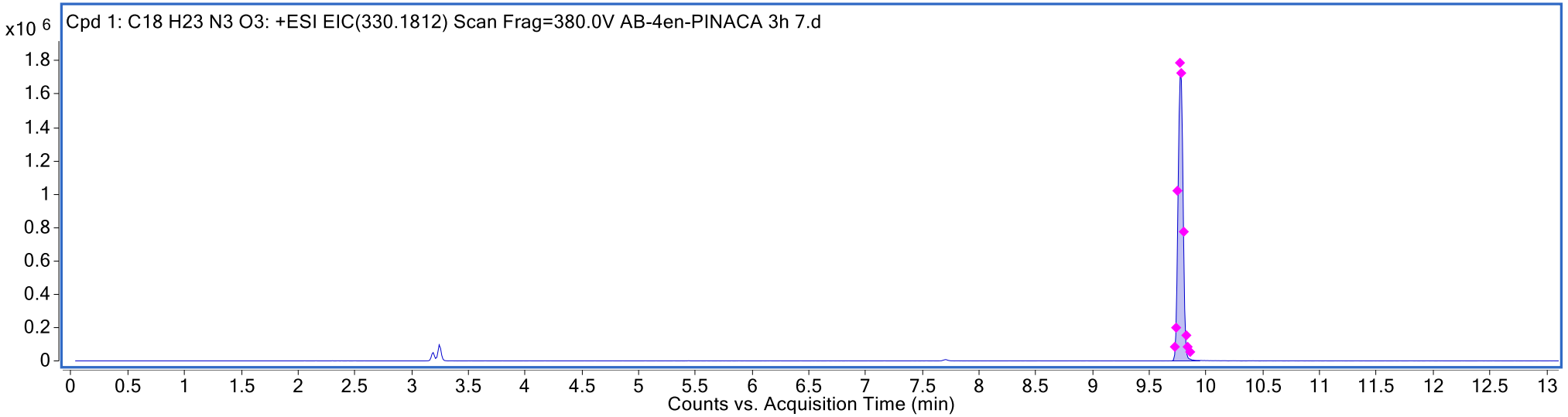


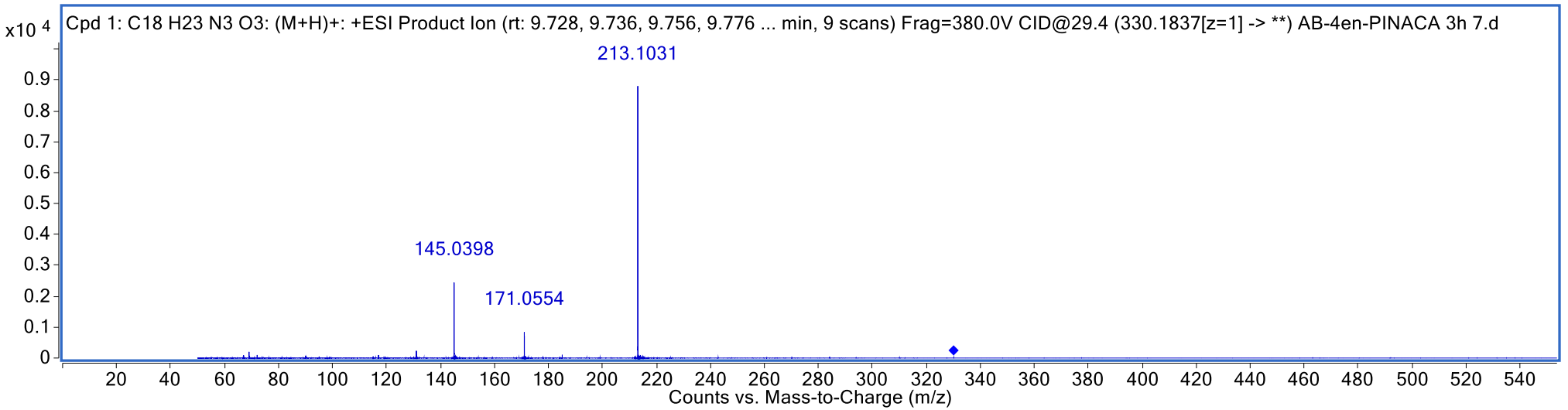
213.1022

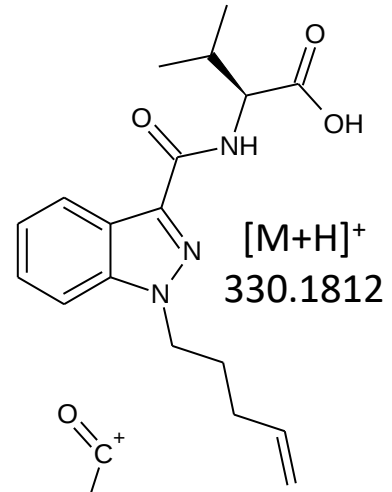


145.0396

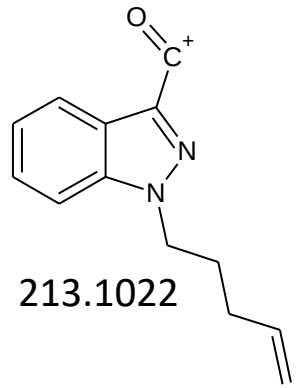
H1, Terminal amide hydrolysis, RT 9.77 min, m/z 330.1840







[M+H]+
330.1812



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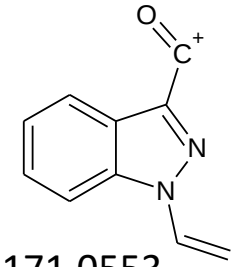
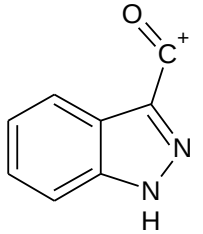
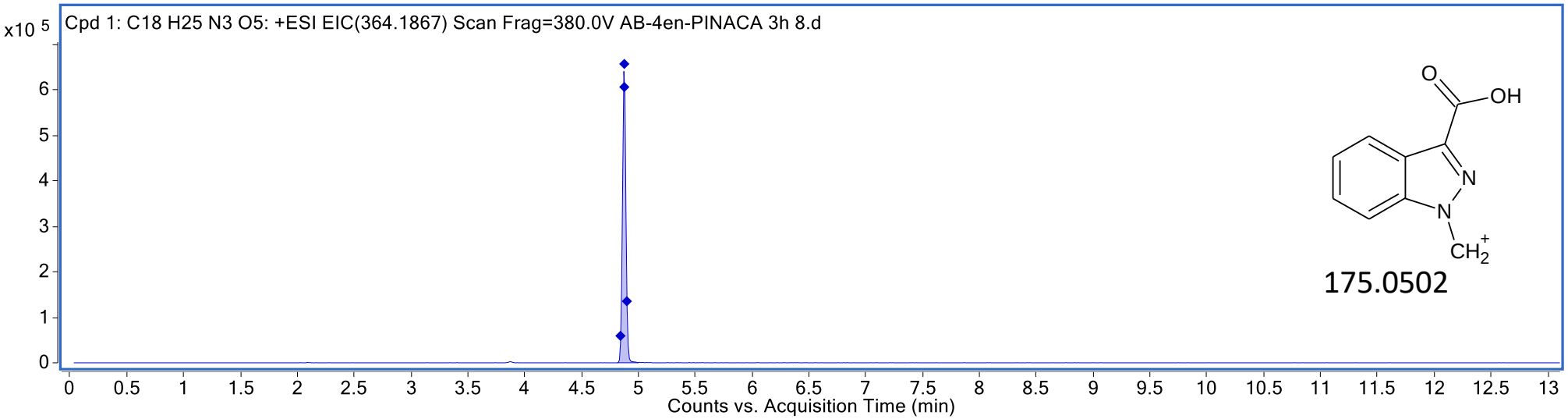


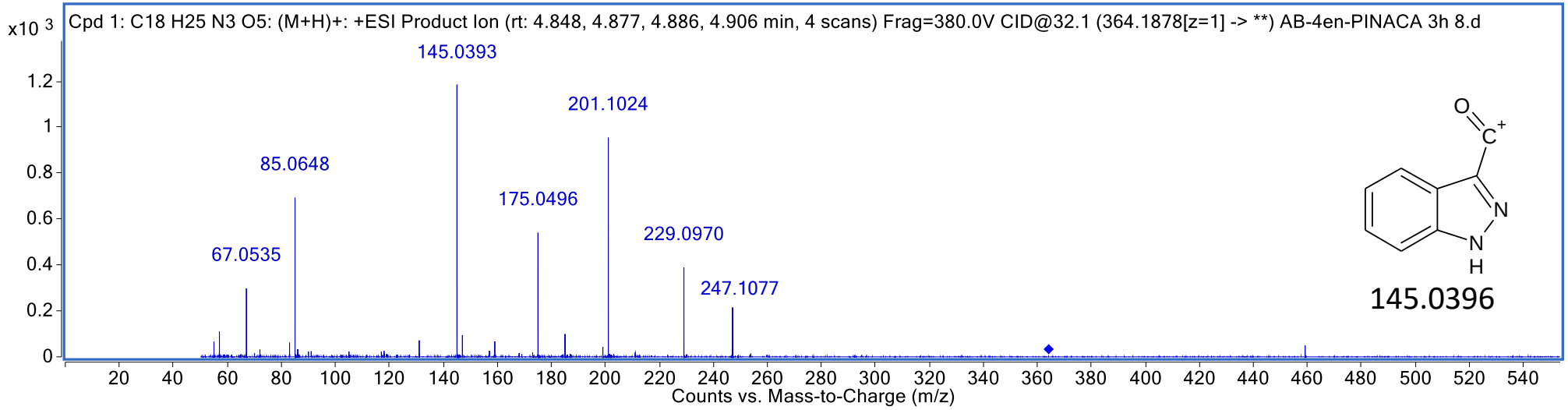
171.0553

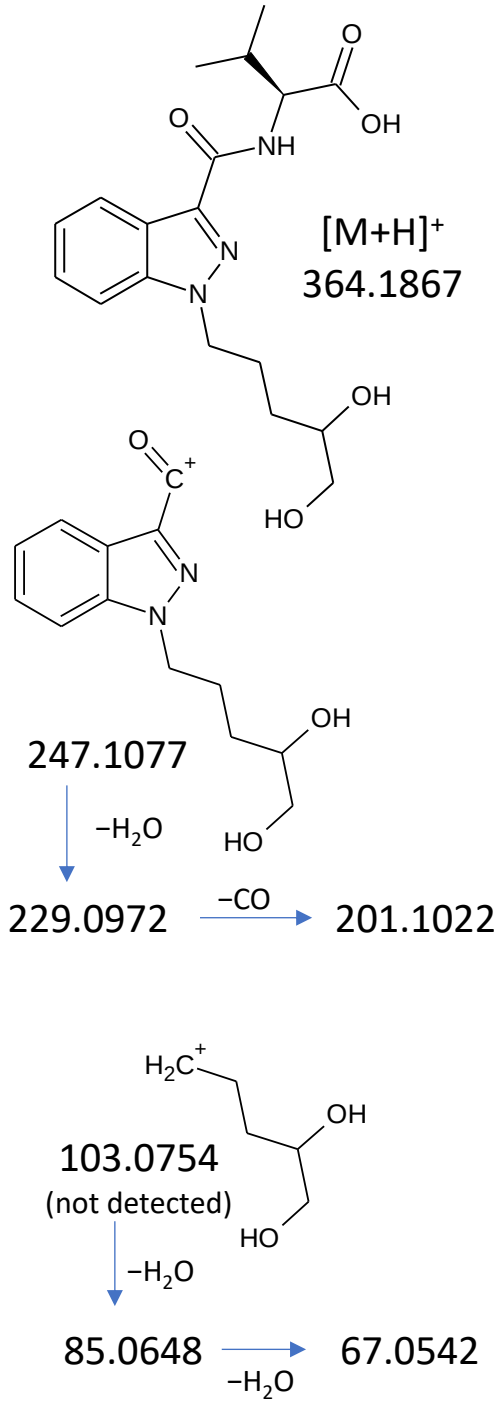


145.0396

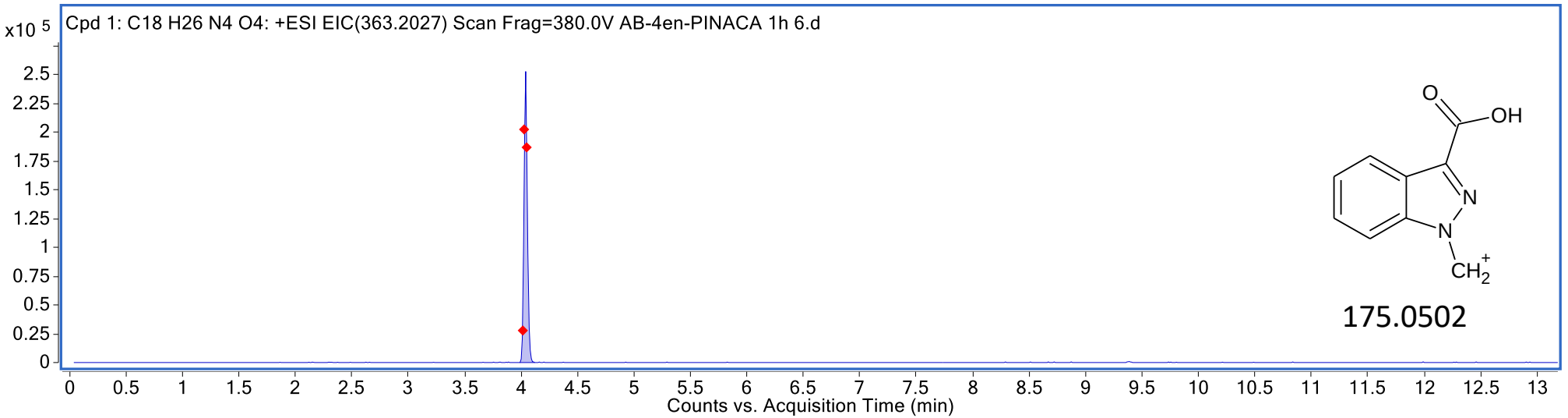
H2, Terminal amide hydrolysis + dihydrodiol formation, RT 4.88 min, *m/z* 364.1877

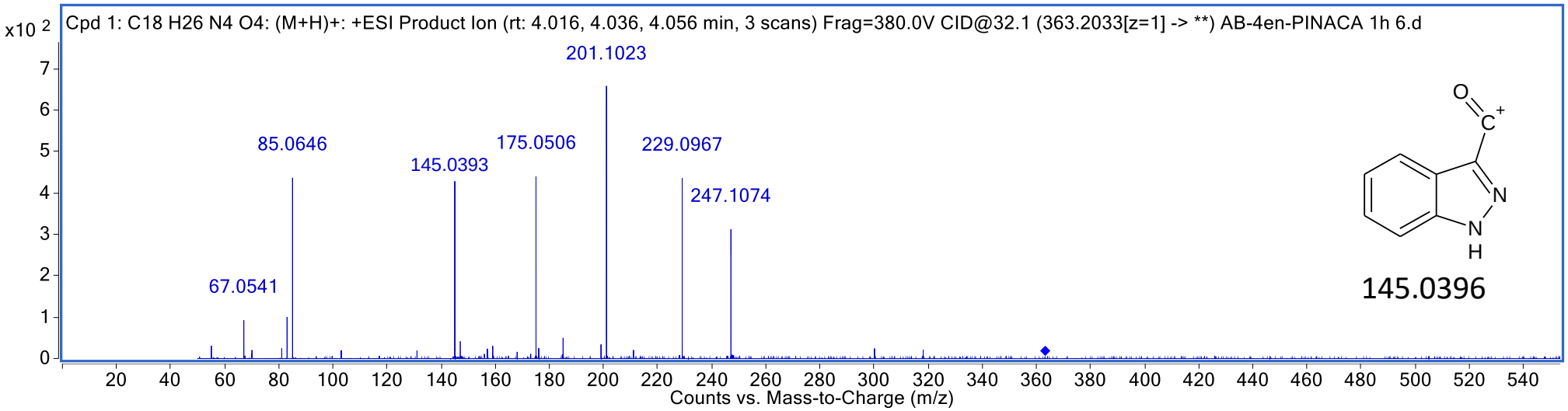


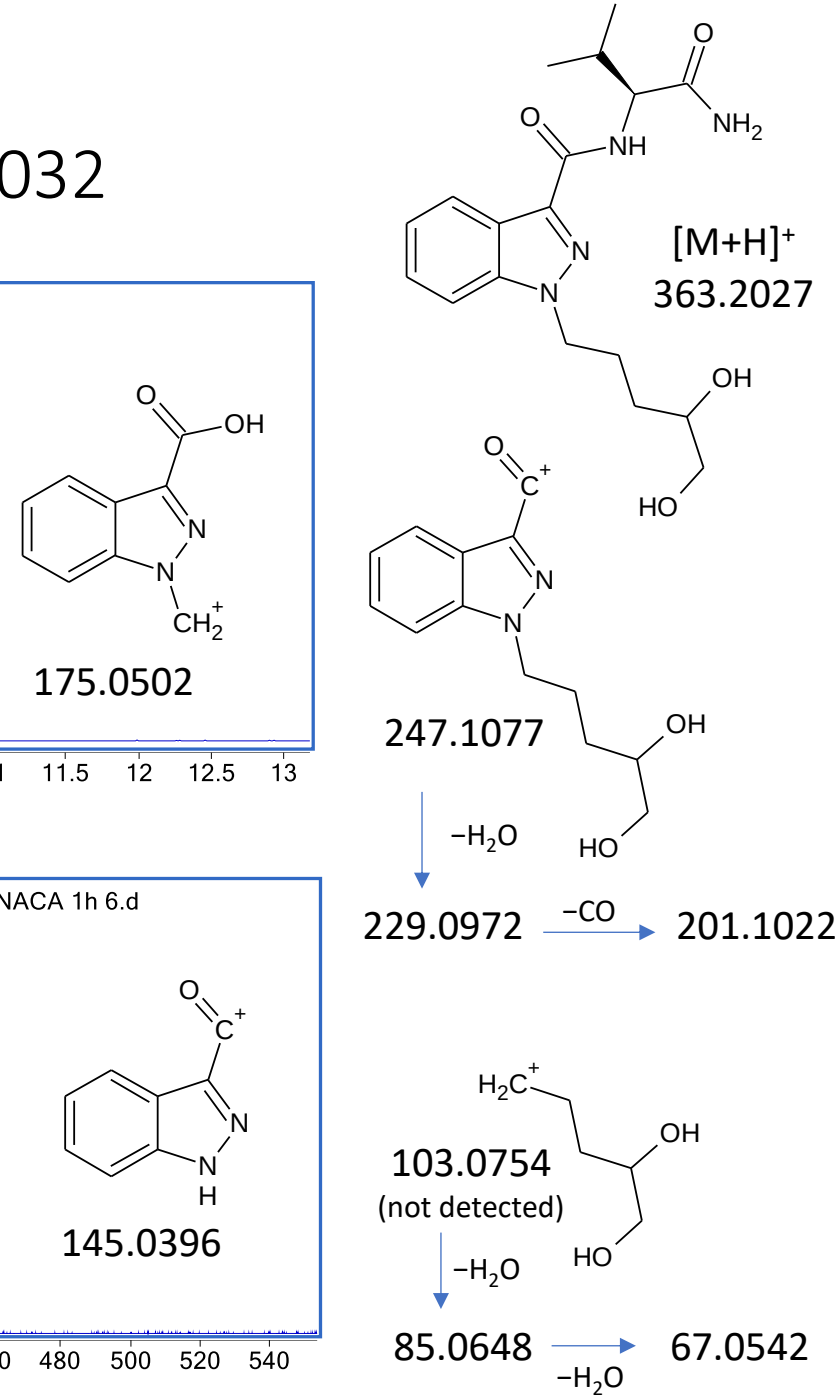




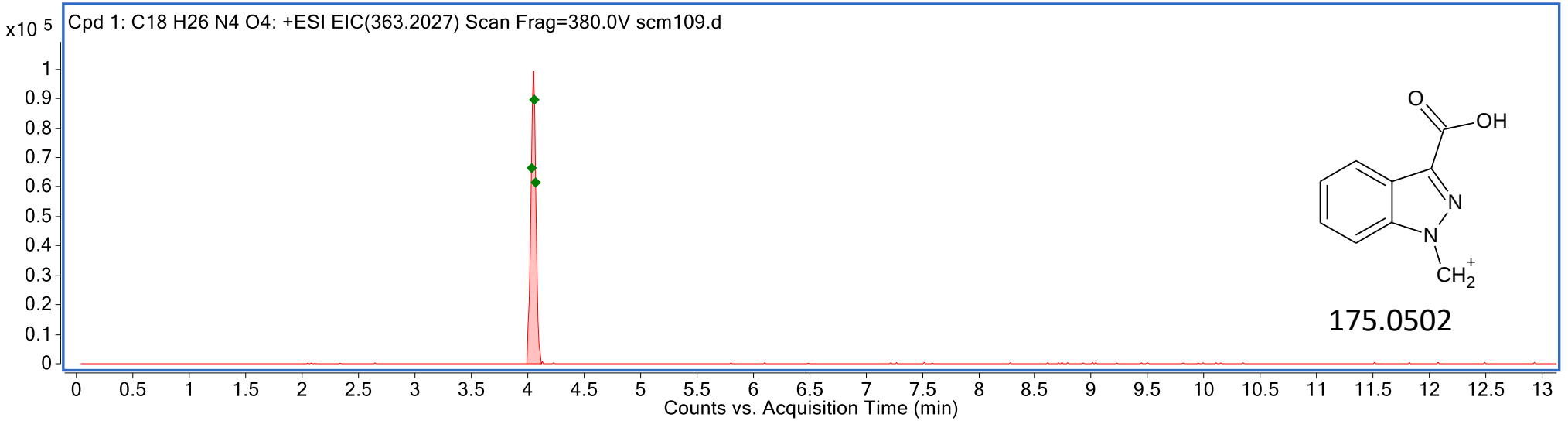
H3, Dihydrodiol formation, RT 4.04 min, *m/z* 363.2032

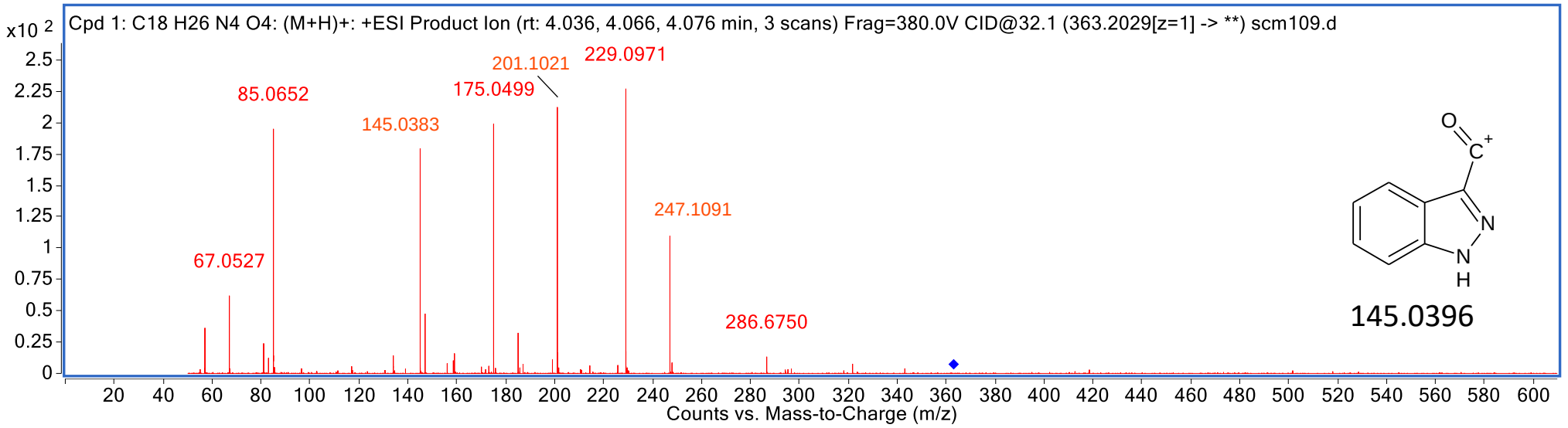




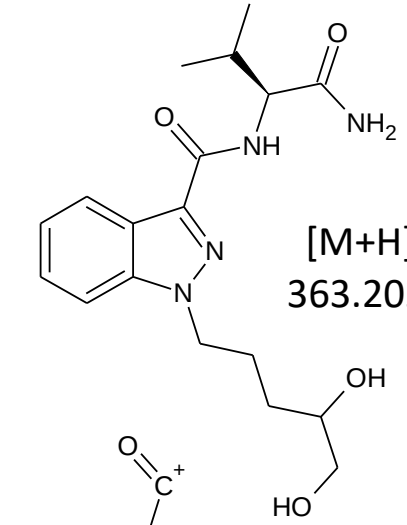


Dihydrodiol reference standard, RT 4.05 min, *m/z* 363.2029

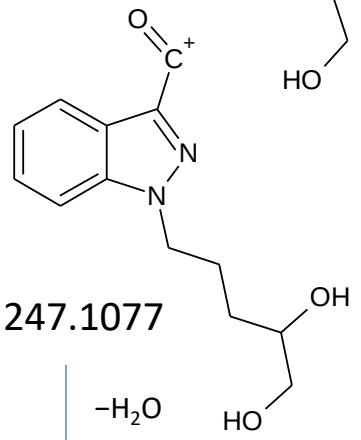




175.0502



[M+H]+
363.2027

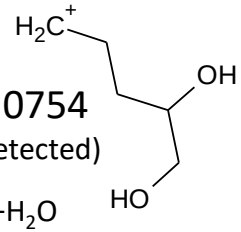


247.1077

$-H_2O$

229.0972 $-CO$ 201.1022

145.0396

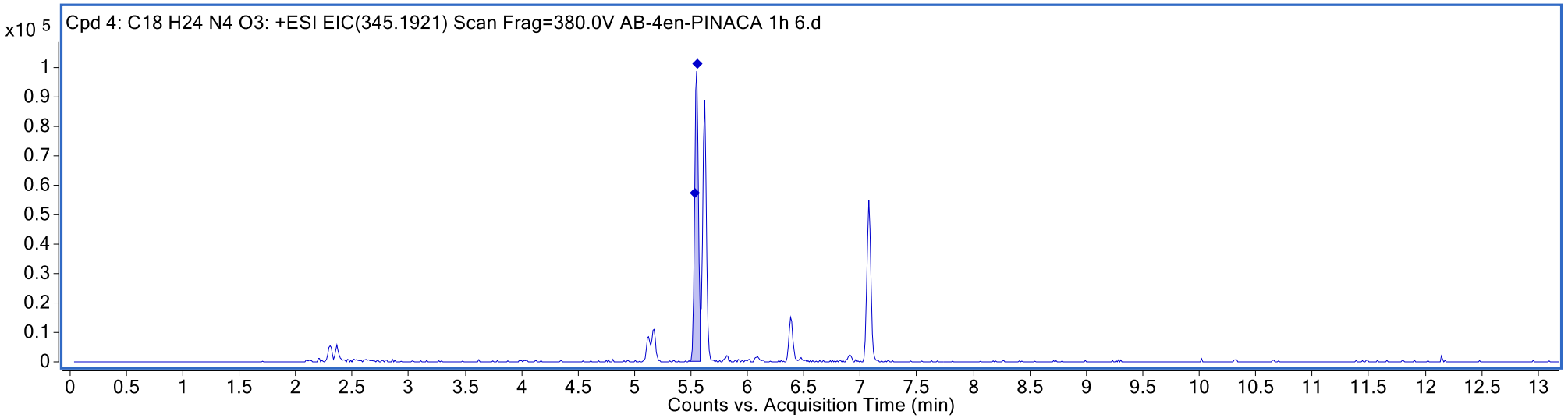


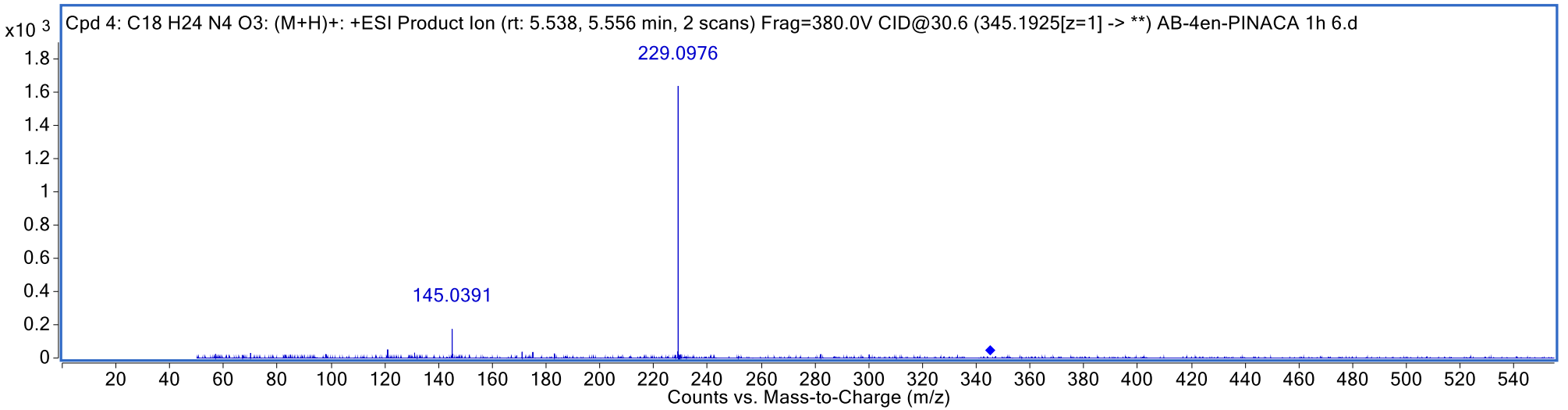
103.0754
(not detected)

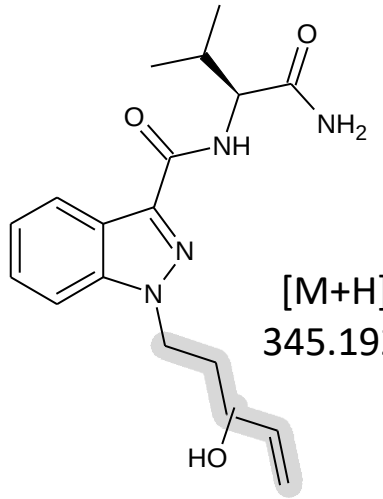
$-H_2O$

85.0648 $-H_2O$ 67.0542

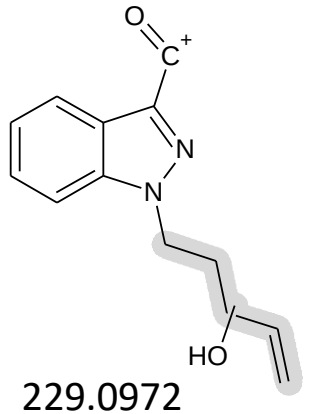
H4, Mono-hydroxylation (pentenyl tail), RT 5.55 min, *m/z* 345.1923



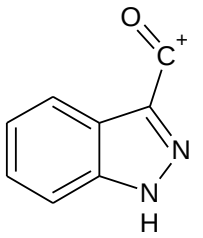




[M+H]+
345.1921

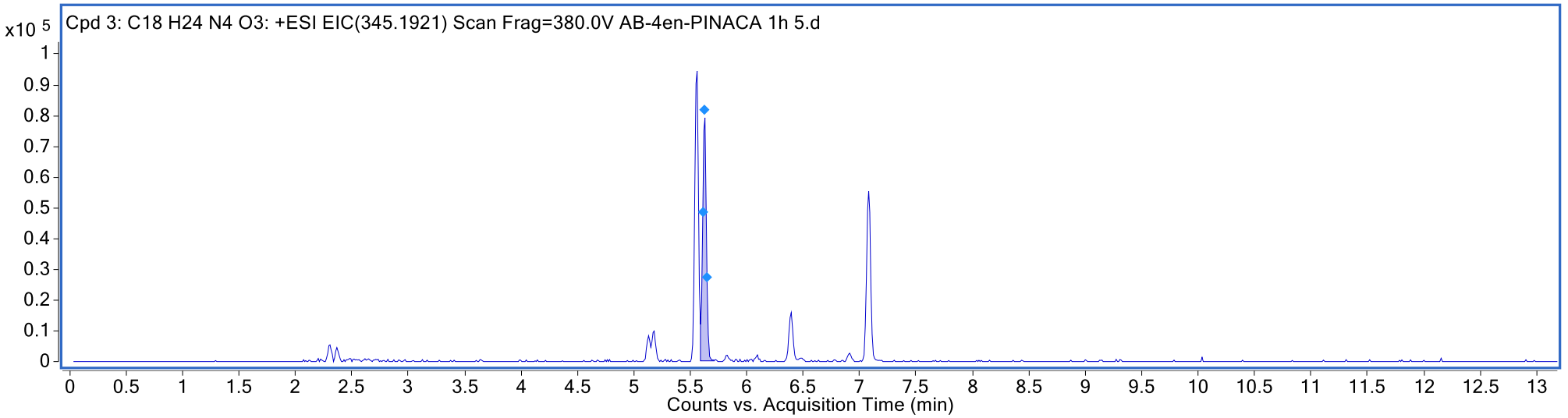


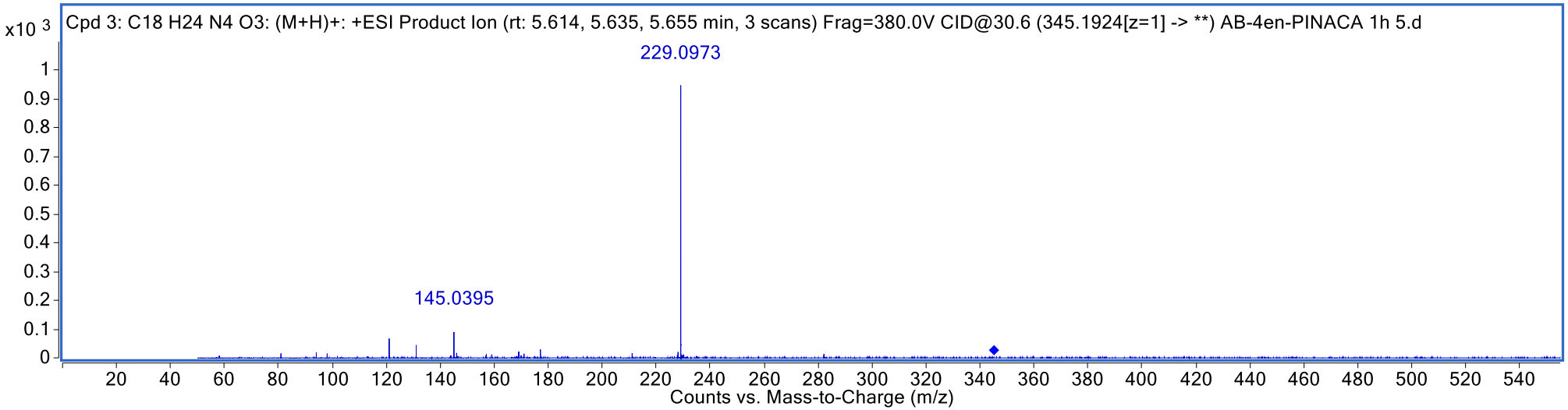
229.0972

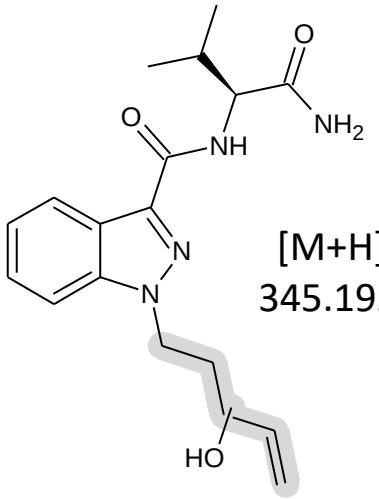


145.0396

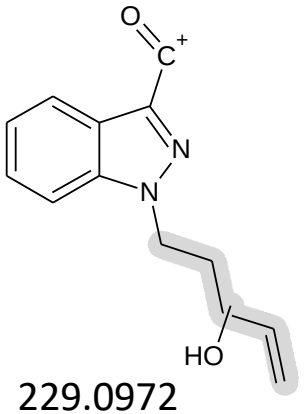
H5, Mono-hydroxylation (pentenyl tail), RT 5.62 min, *m/z* 345.1920



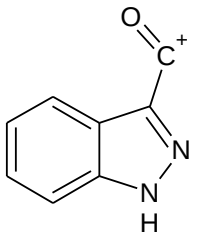




$[M+H]^+$
345.1921

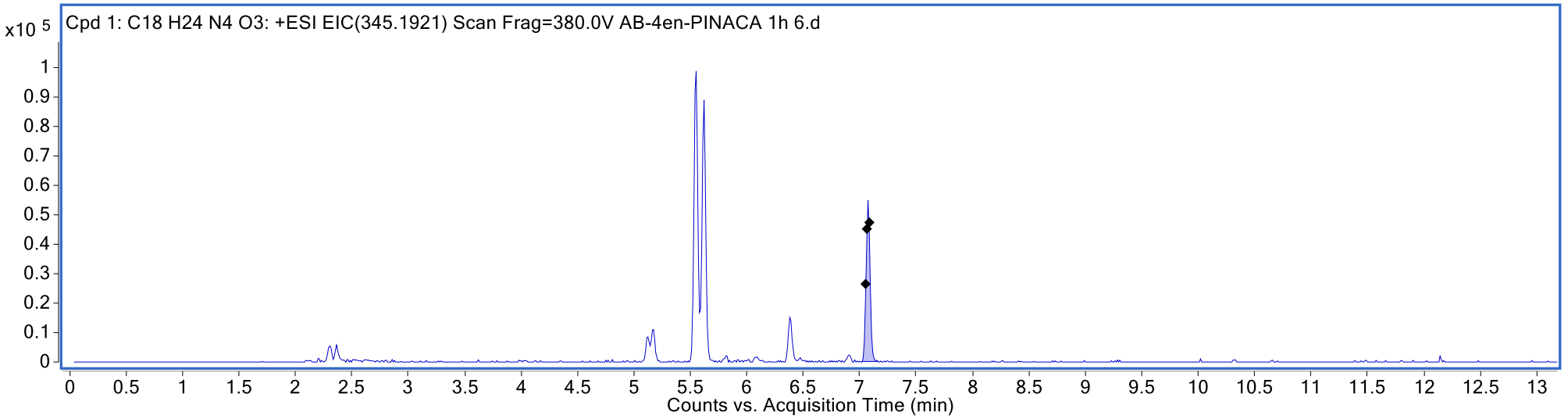


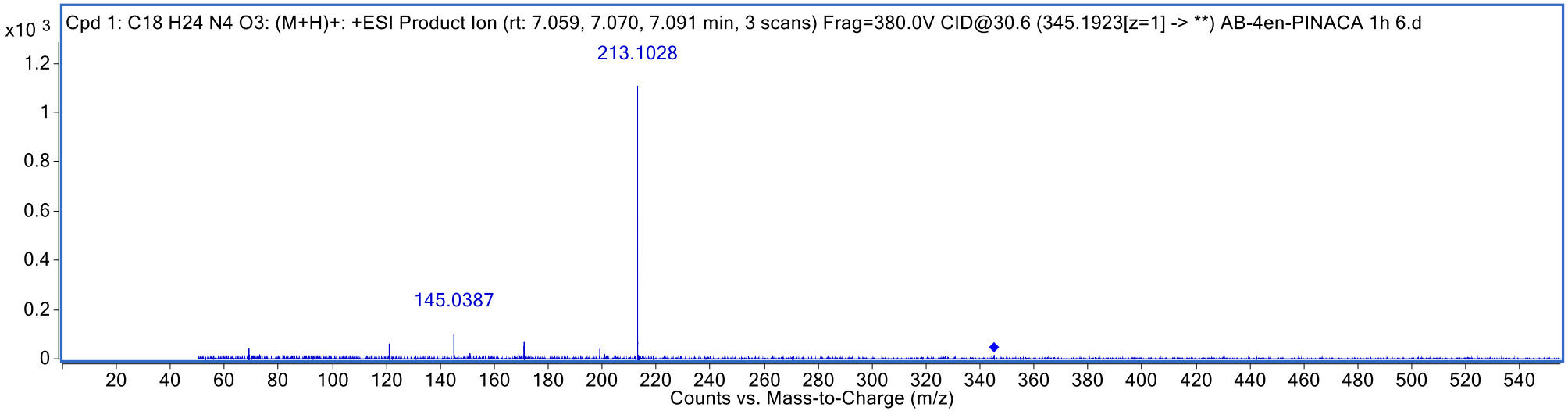
229.0972

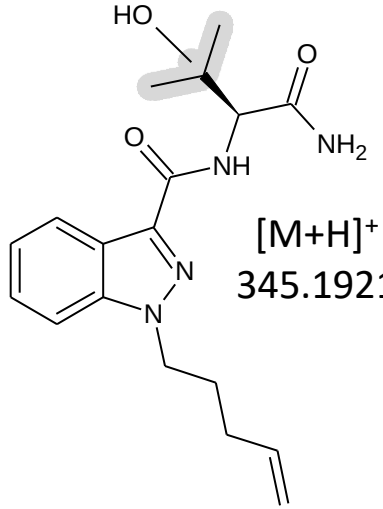


145.0396

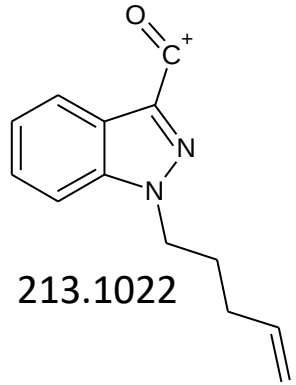
H6, Mono-hydroxylation (*iso*-propyl), RT 7.08 min, *m/z* 345.1924



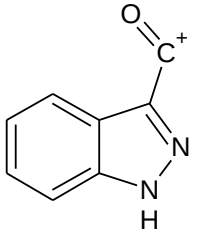




[M+H]+
345.1921

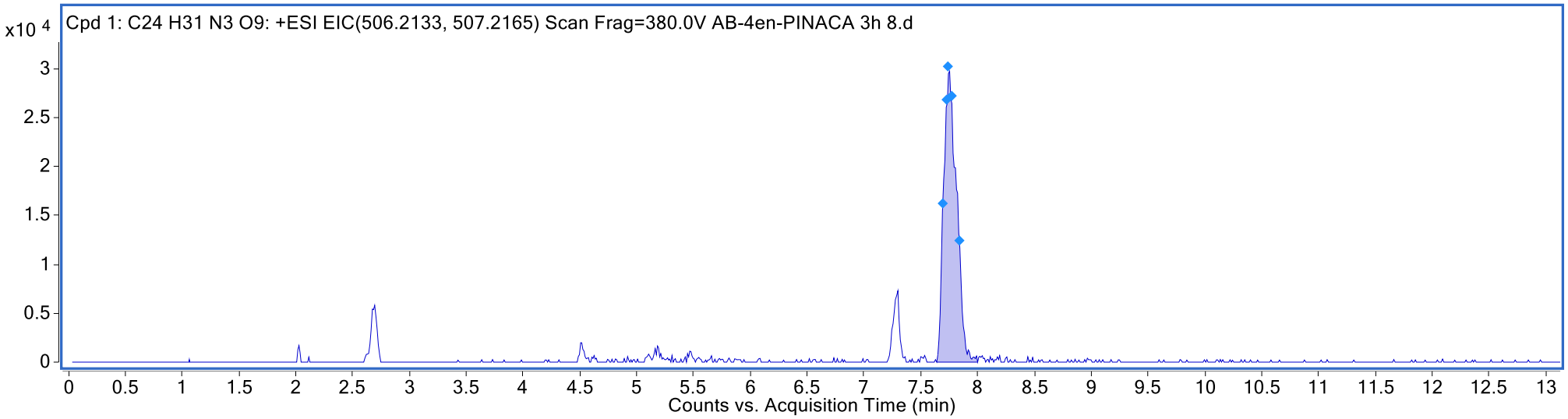


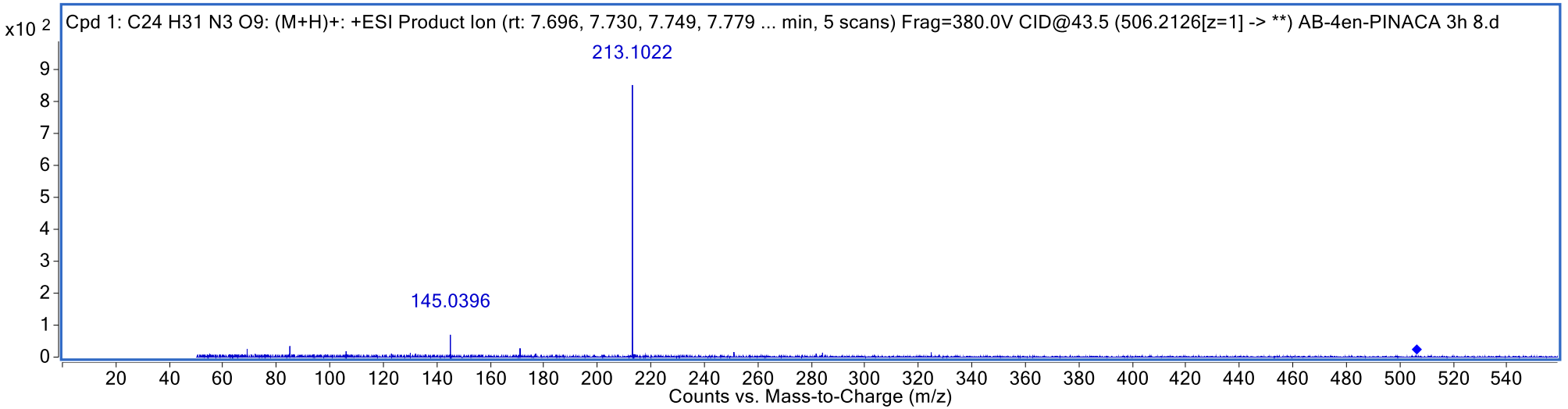
213.1022

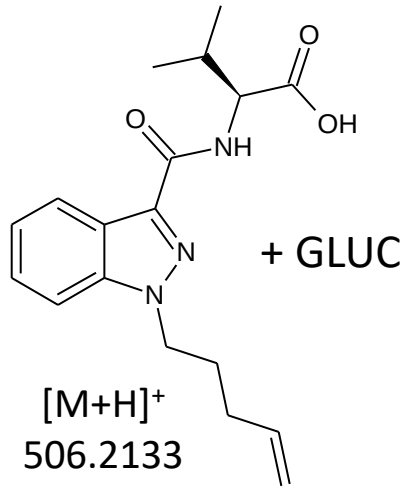


145.0396

H7, Terminal amide hydrolysis + glucuronidation, RT 7.70 min, *m/z* 506.2126

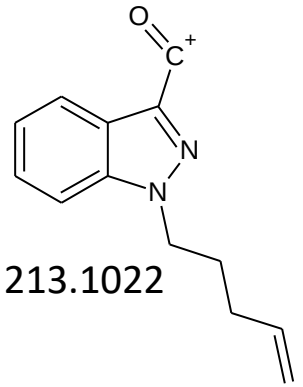




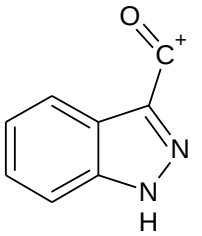


+ GLUC

$[M+H]^+$
506.2133

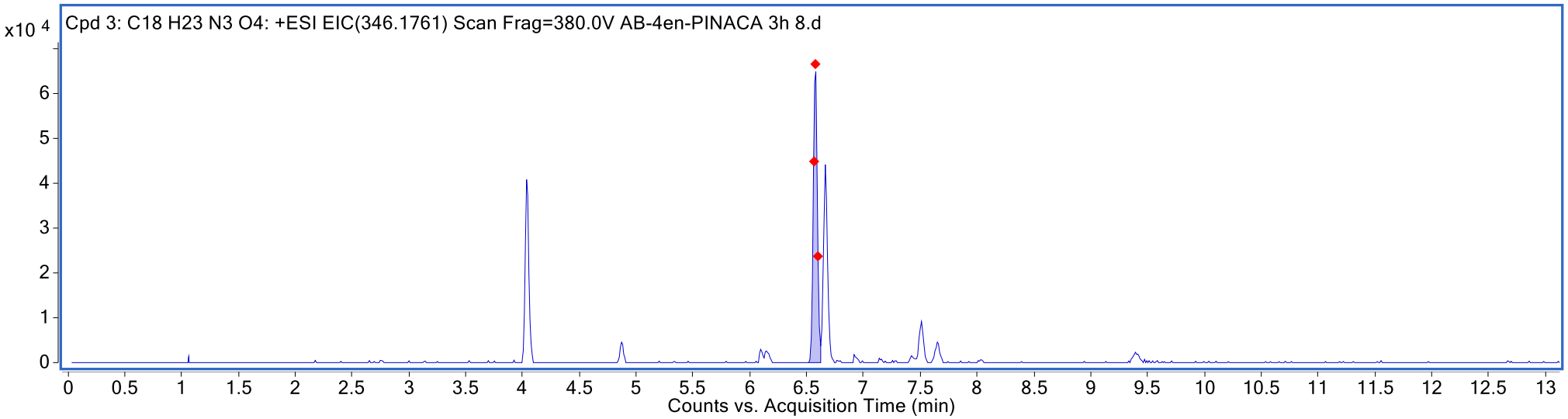


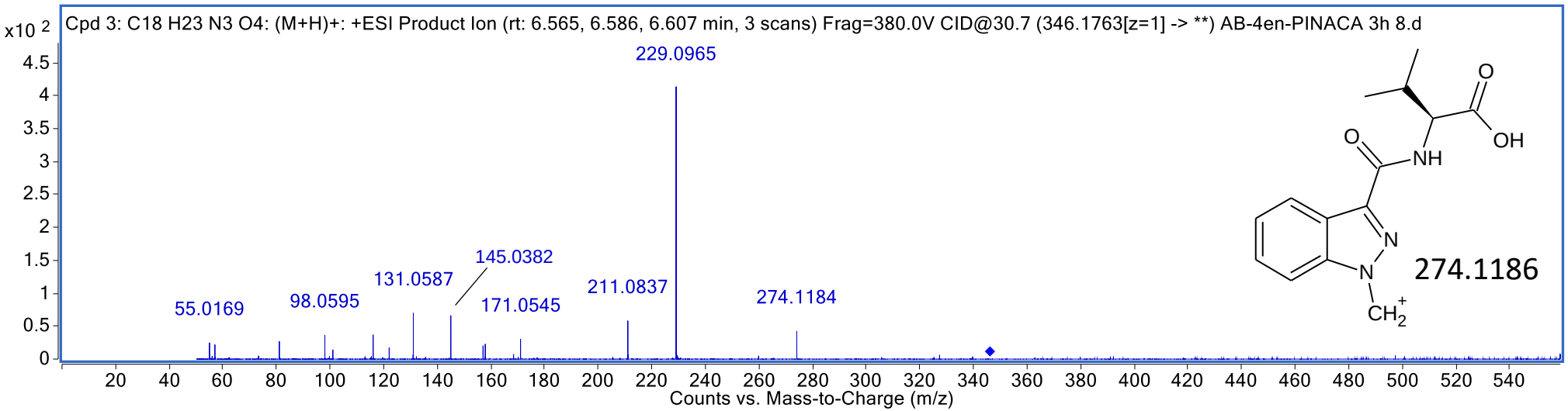
213.1022

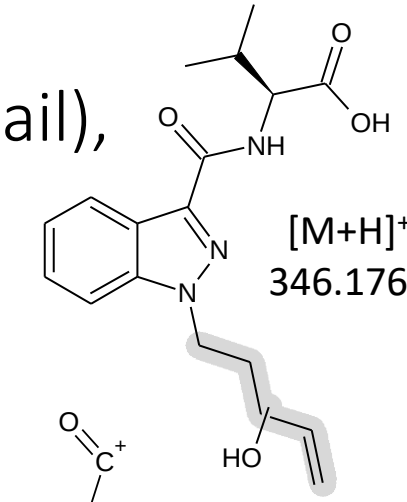


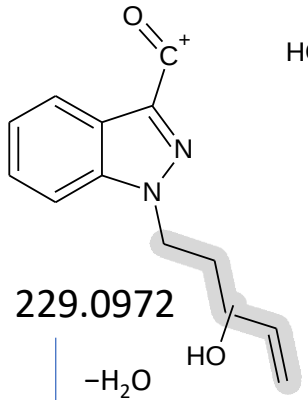
145.0396

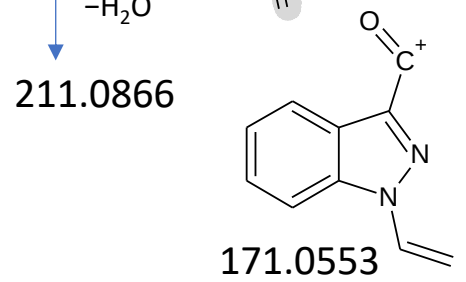
H8, Terminal amide hydrolysis + mono-hydroxylation (pentenyl tail), RT 6.58 min, *m/z* 346.1762

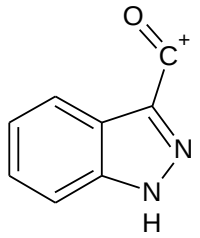


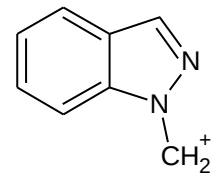




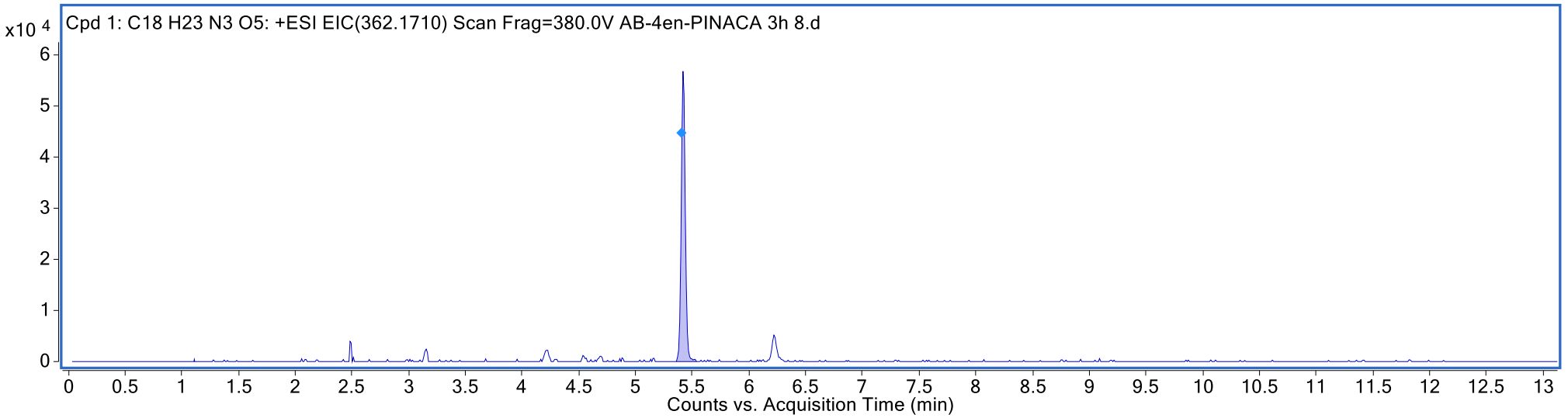


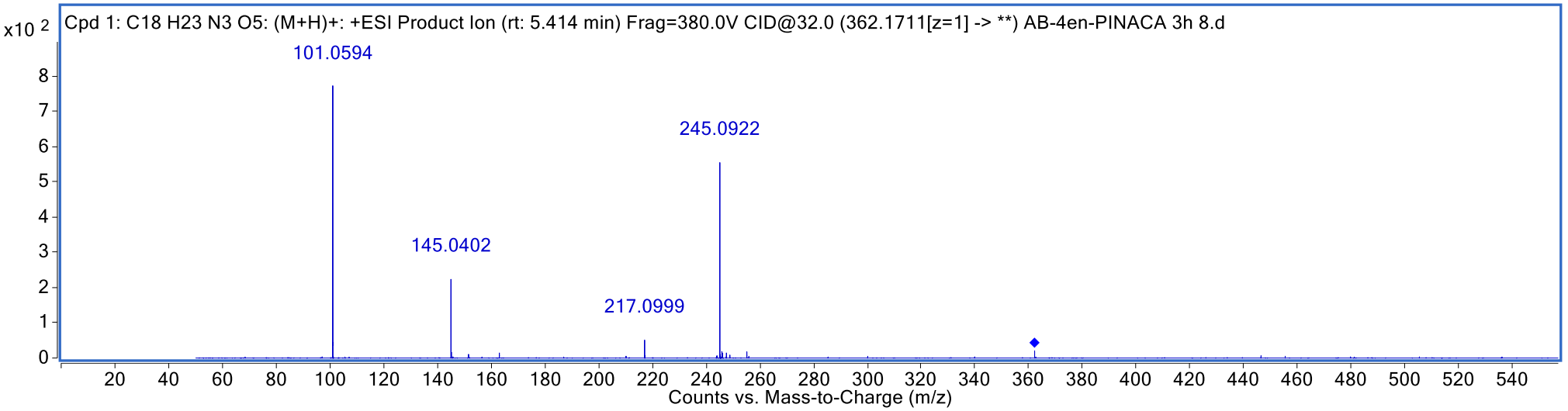


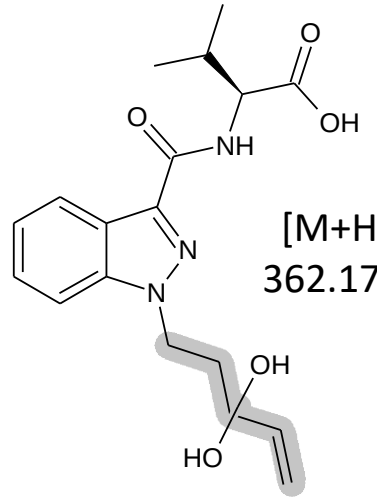


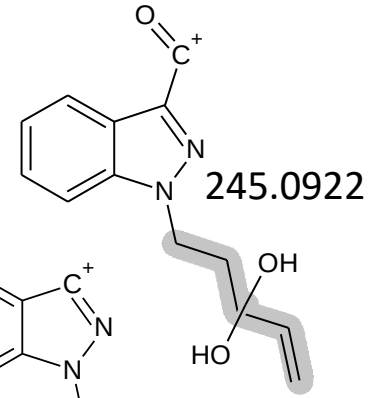


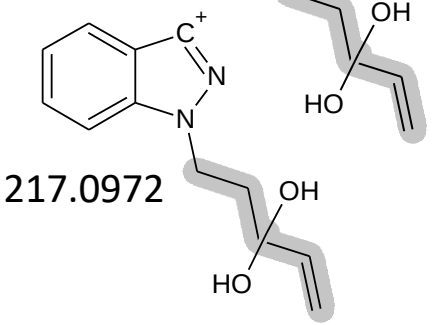
H9, Terminal amide hydrolysis + di-hydroxylation (pentenyl tail), RT 5.42 min, *m/z* 362.1704

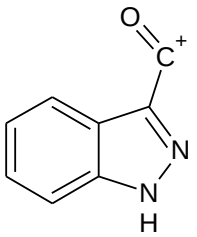


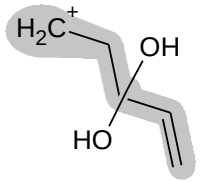




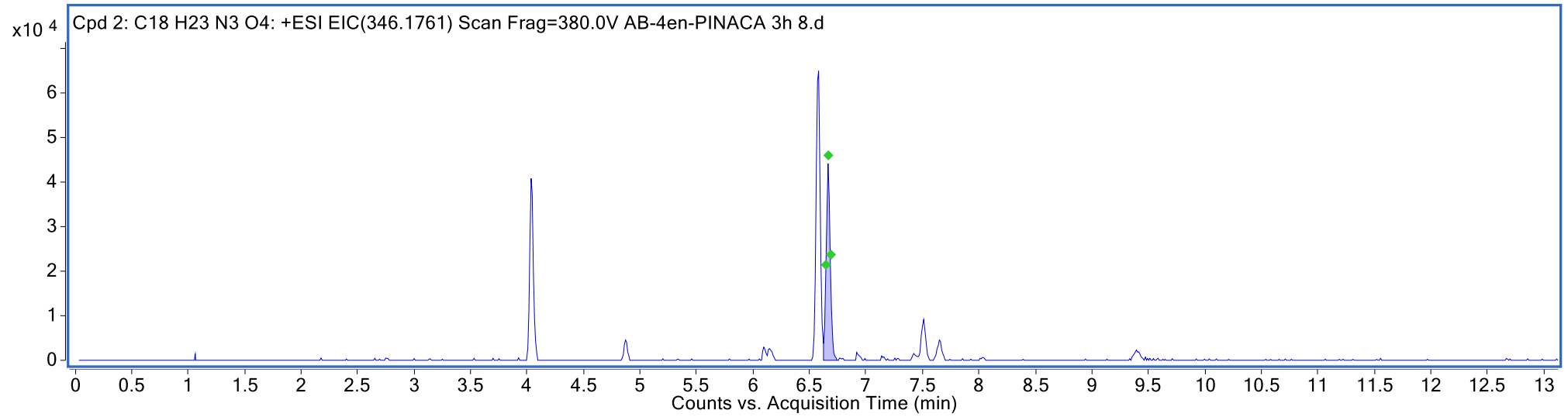


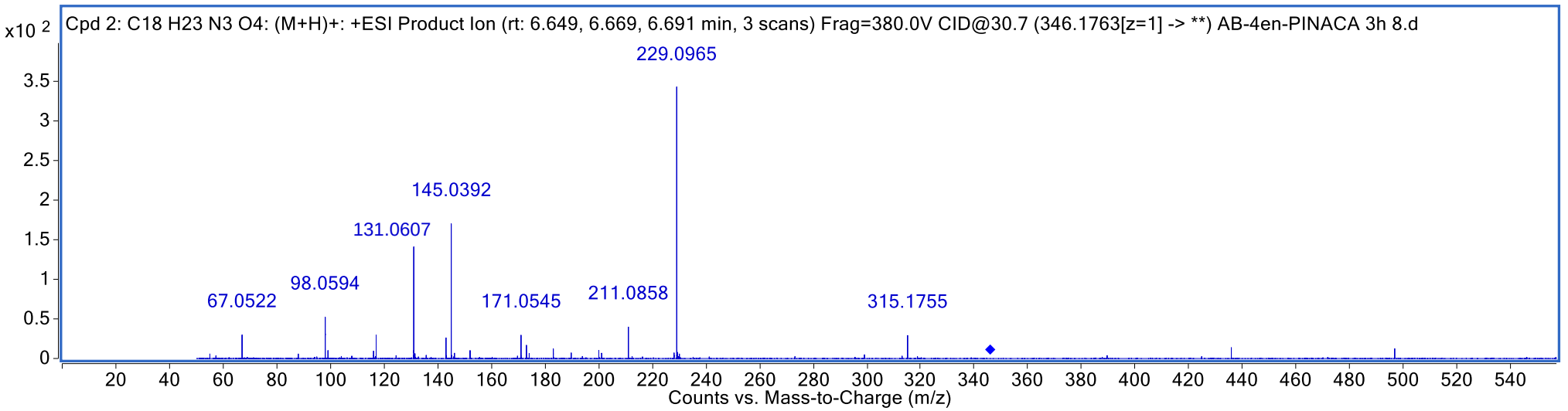


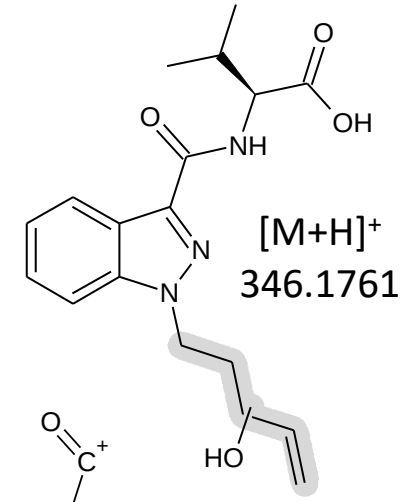


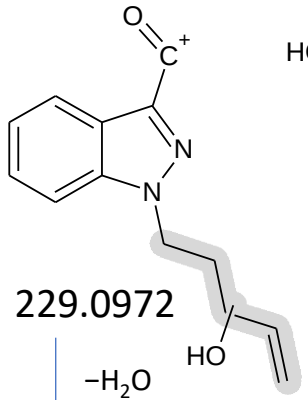


H10, Terminal amide hydrolysis + mono-hydroxylation (pentenyl tail), RT 6.67 min, *m/z* 346.1764



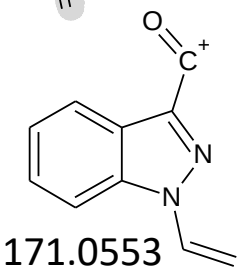


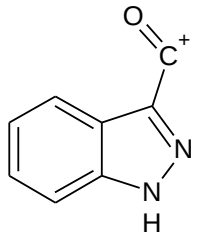


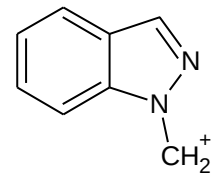


$-H_2O$

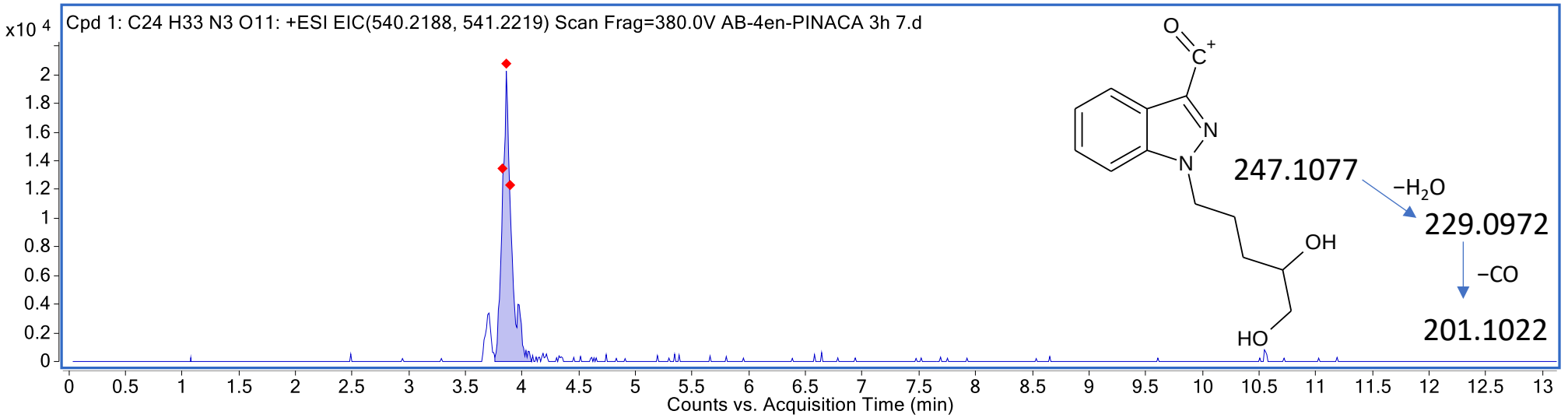
211.0866

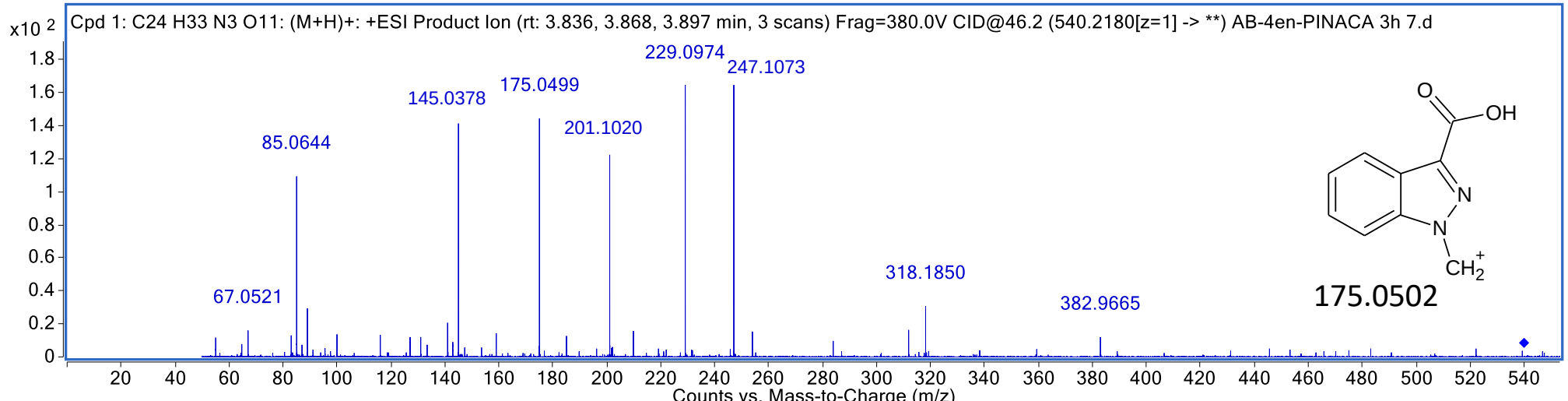


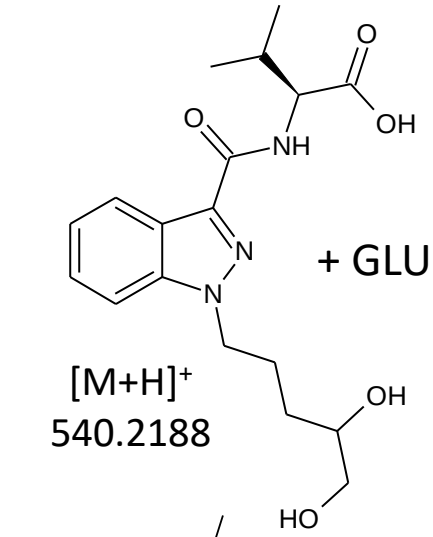




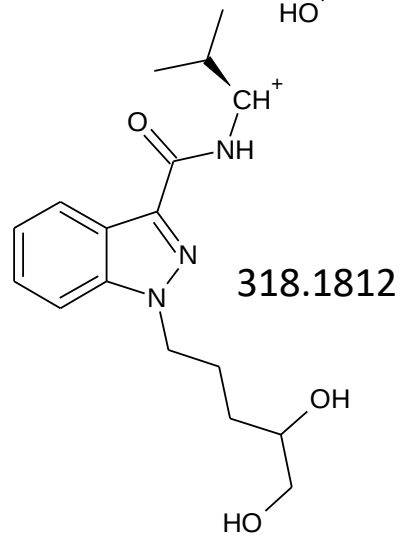
H11, Terminal amide hydrolysis + dihydrodiol formation + glucuronidation, RT 3.87 min, *m/z* 540.2180

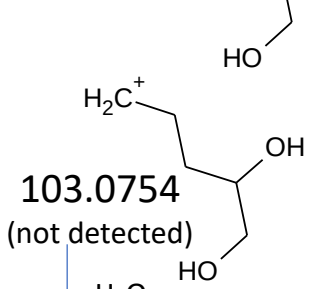


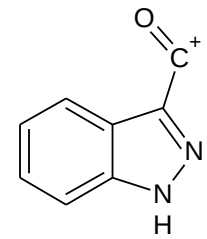




247.1077 $-H_2O$ 229.0972 $-CO$ 201.1022



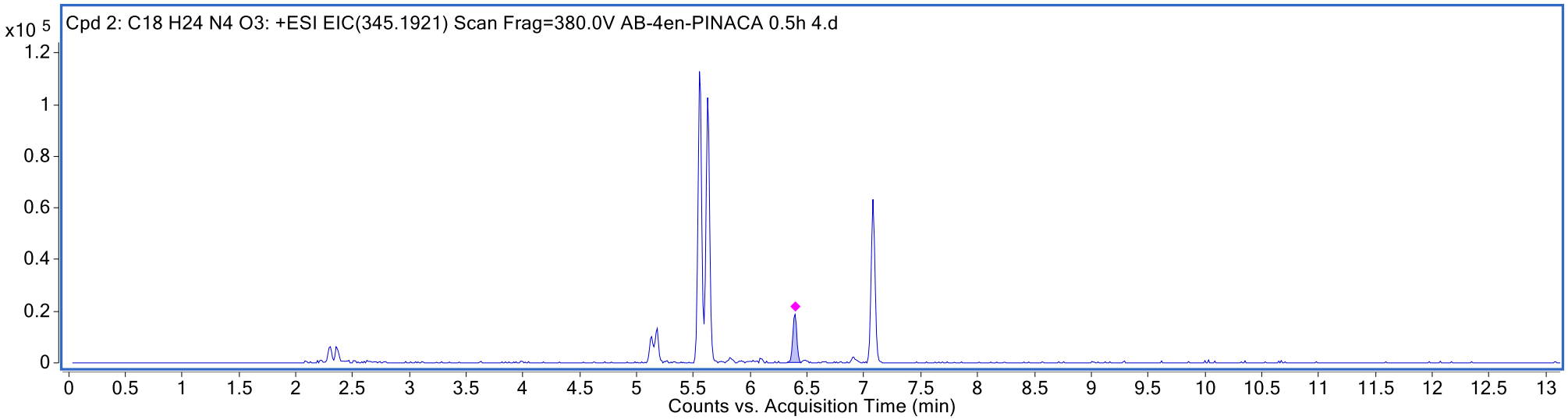


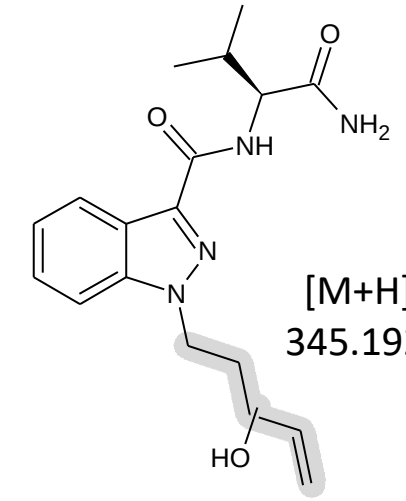


$-H_2O$

85.0648 $-H_2O$ 67.0542

H12, Mono-hydroxylation (pentenyl tail), RT 6.39 min, *m/z* 345.1917





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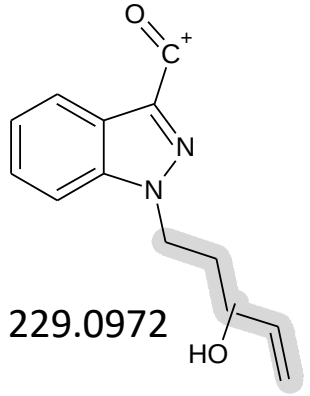
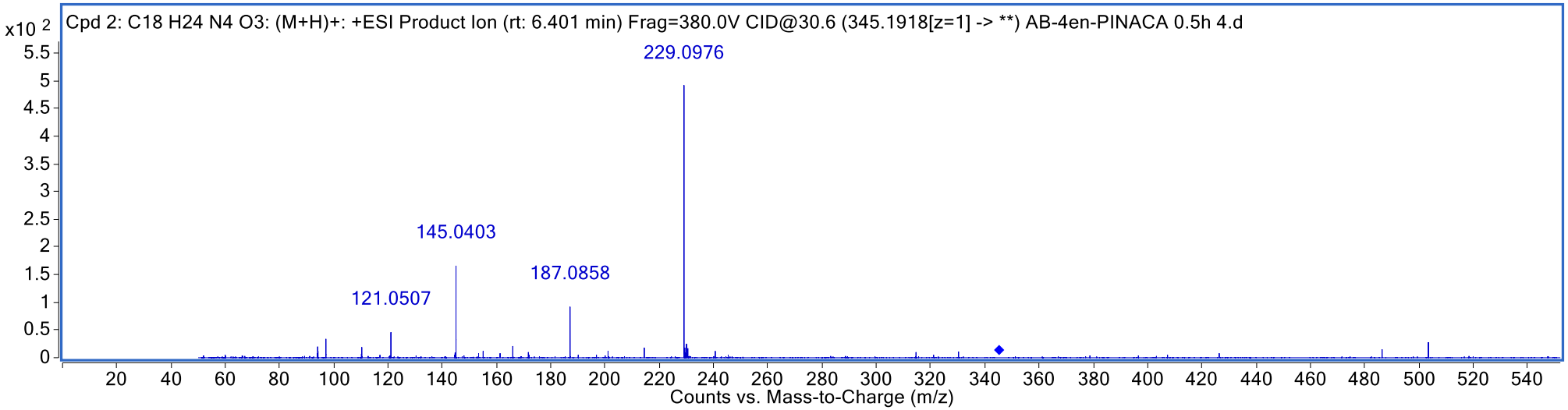
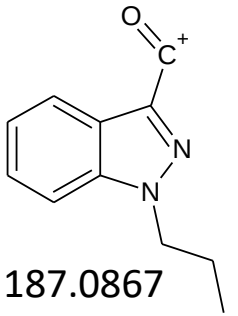
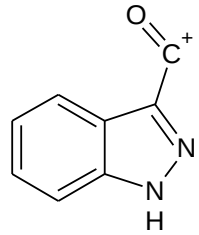


229.0972



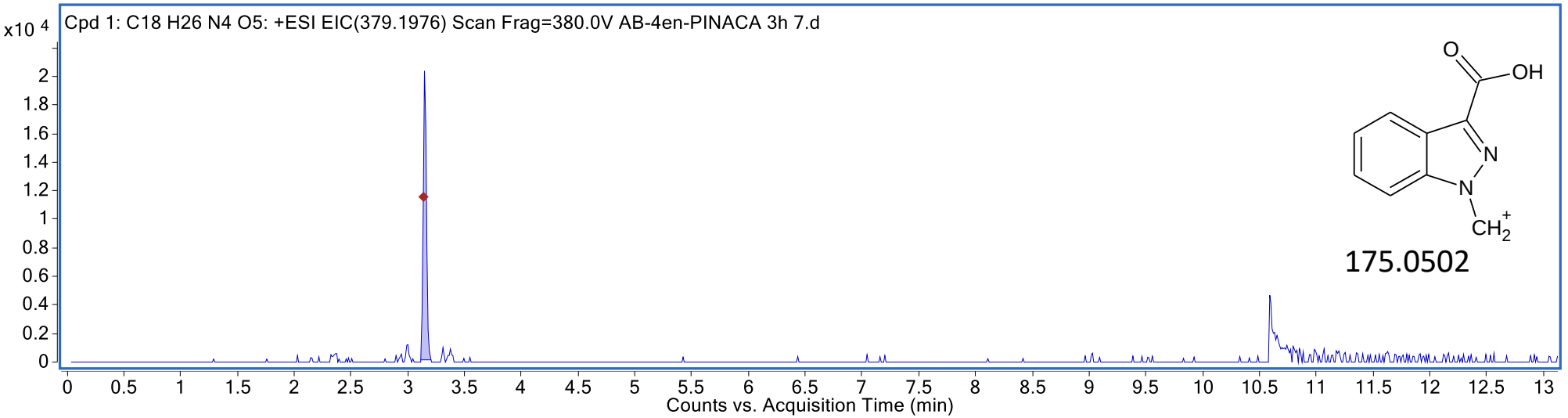


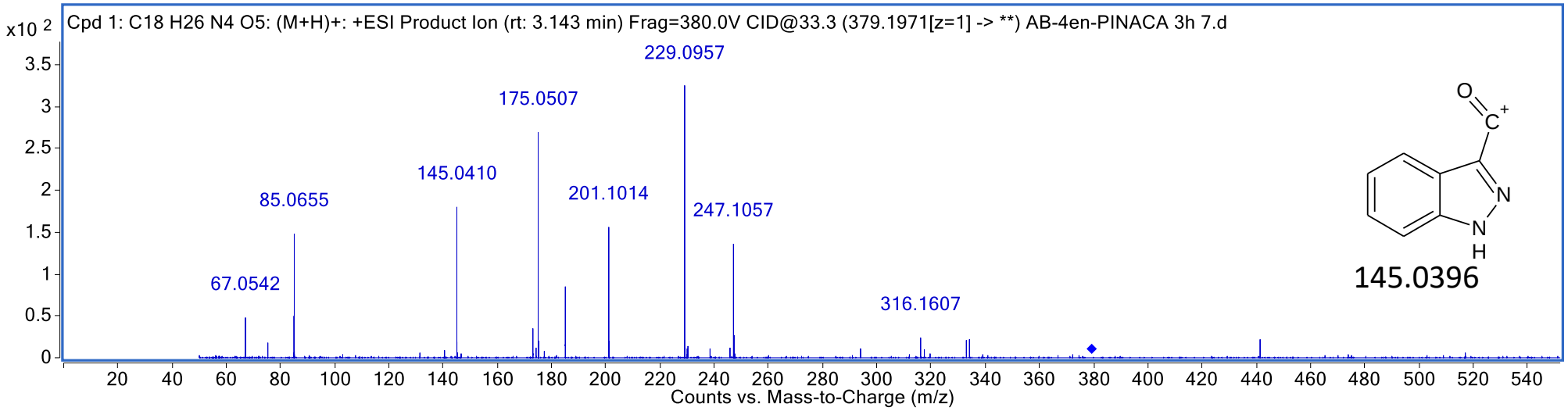
187.0867

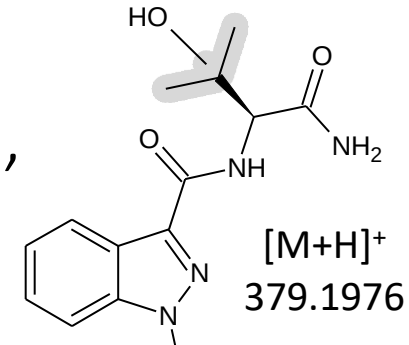


145.0396

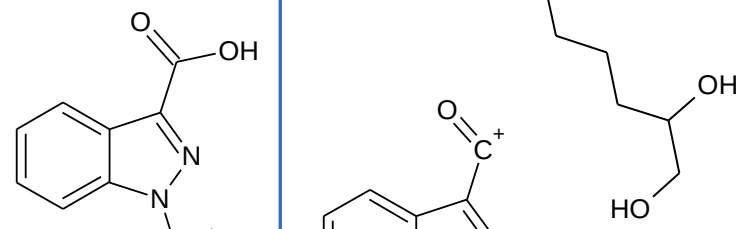
H13, Dihydrodiol + mono-hydroxylation (*iso*-propyl), RT 3.16 min, *m/z* 379.1970



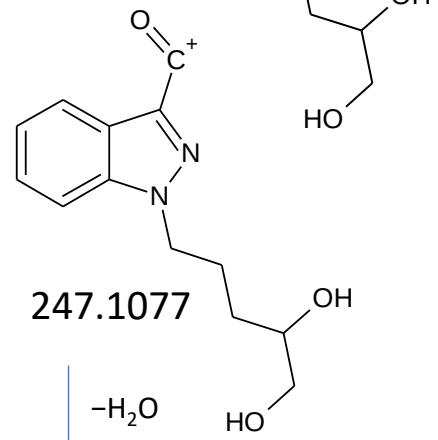




[M+H]+
379.1976



175.0502

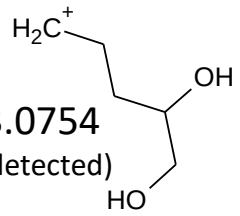


247.1077

$-H_2O$

229.0972 $\xrightarrow{-CO}$ 201.1022

145.0396



103.0754
(not detected)

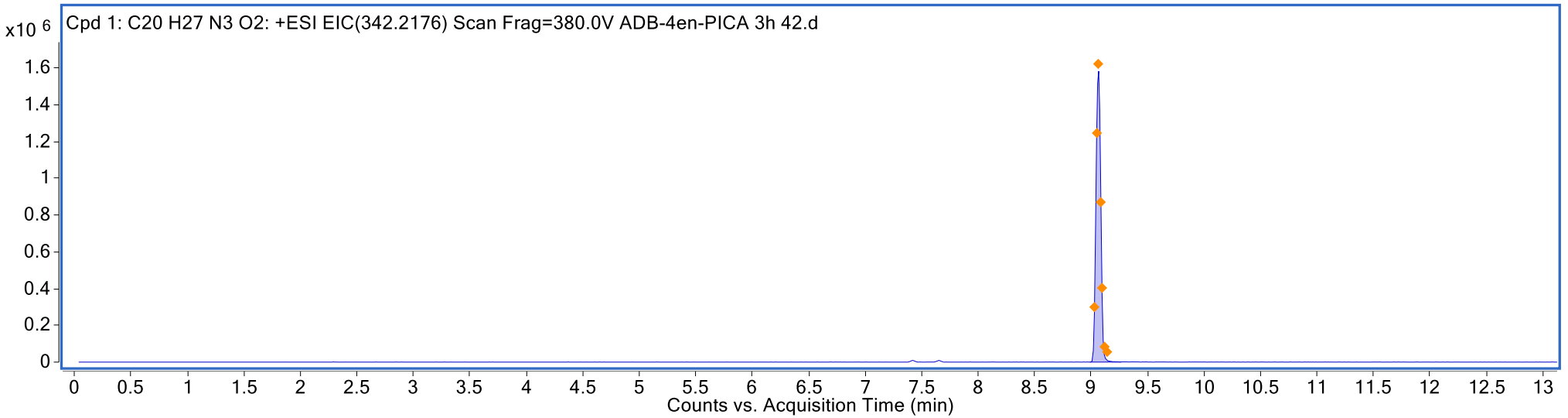
$-H_2O$

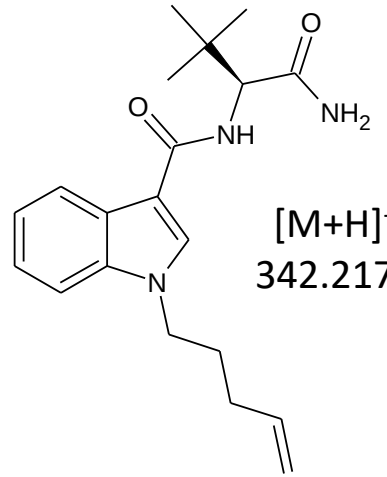
85.0648 $\xrightarrow[-H_2O]{}$ 67.0542

ADB-4en-PICA

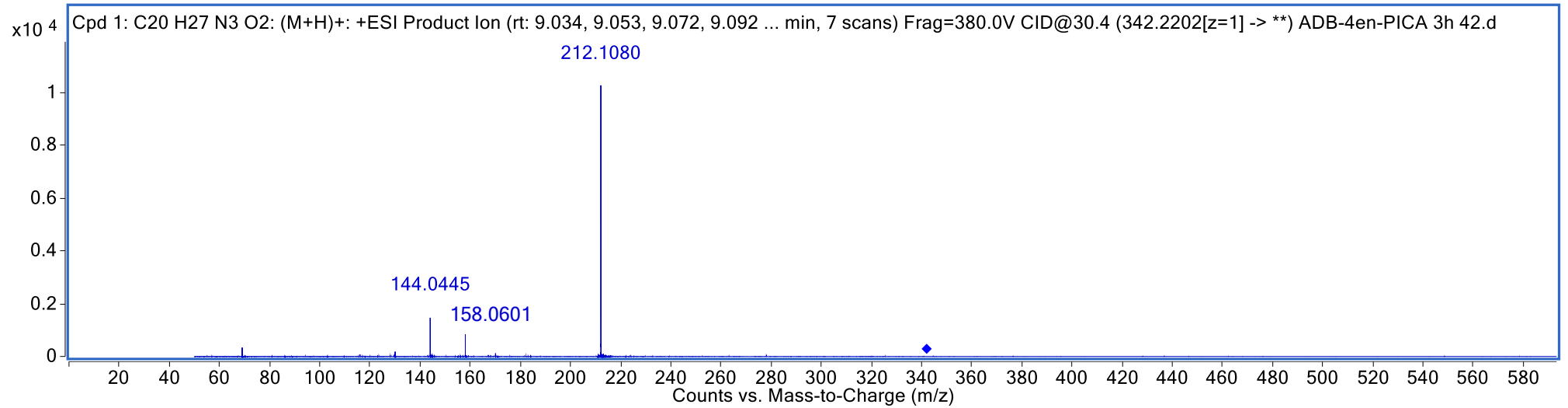
Metabolism

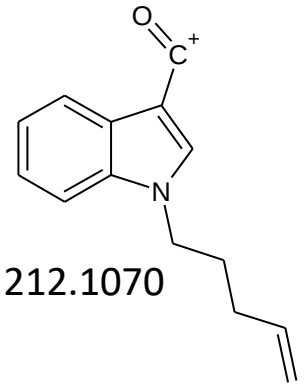
ADB-4en-PICA, RT 9.06 min, *m/z* 342.2210



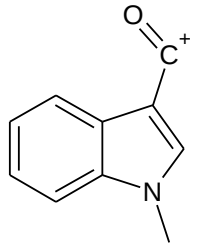


[M+H]+
342.2176

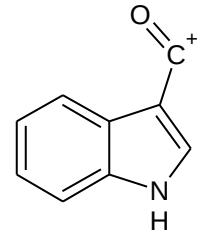




212.1070

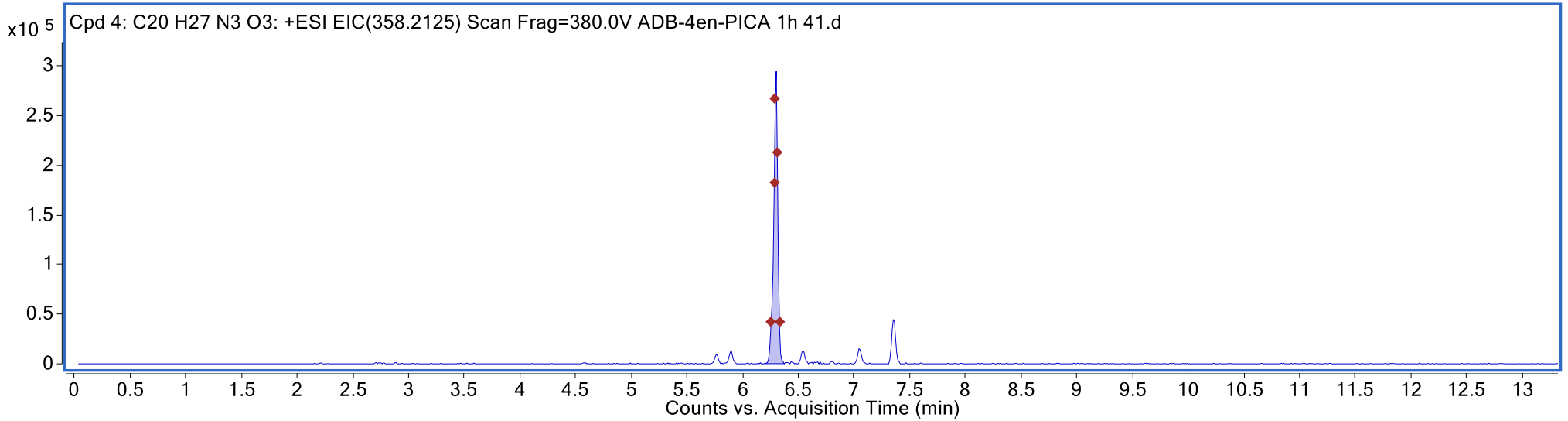


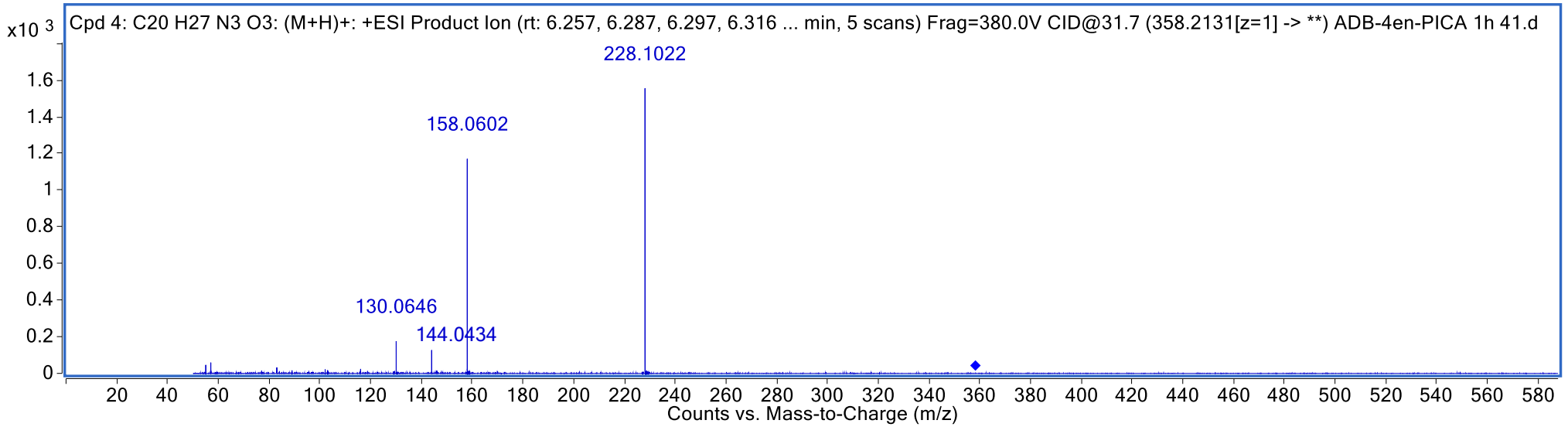
158.0600

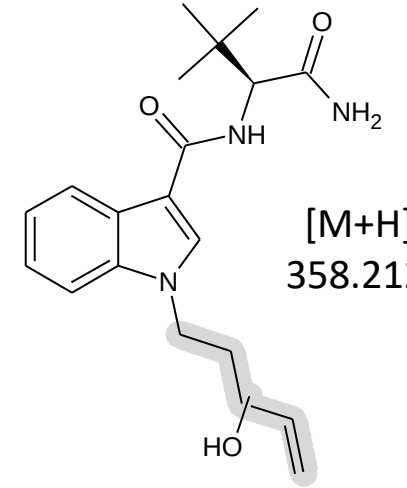


144.0444

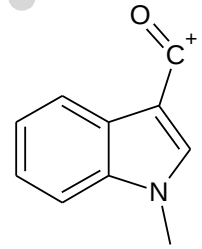
I1, Mono-hydroxylation (pentenyl tail), RT 6.30 min, *m/z* 358.2133



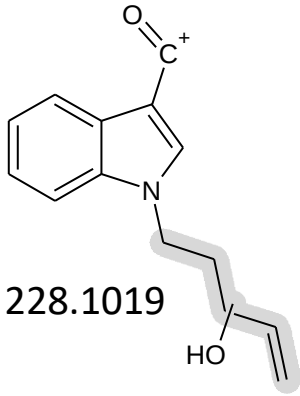




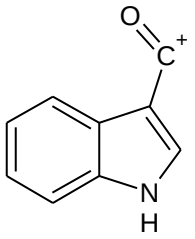
[M+H]+
358.2125



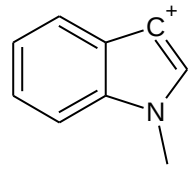
158.0600



228.1019

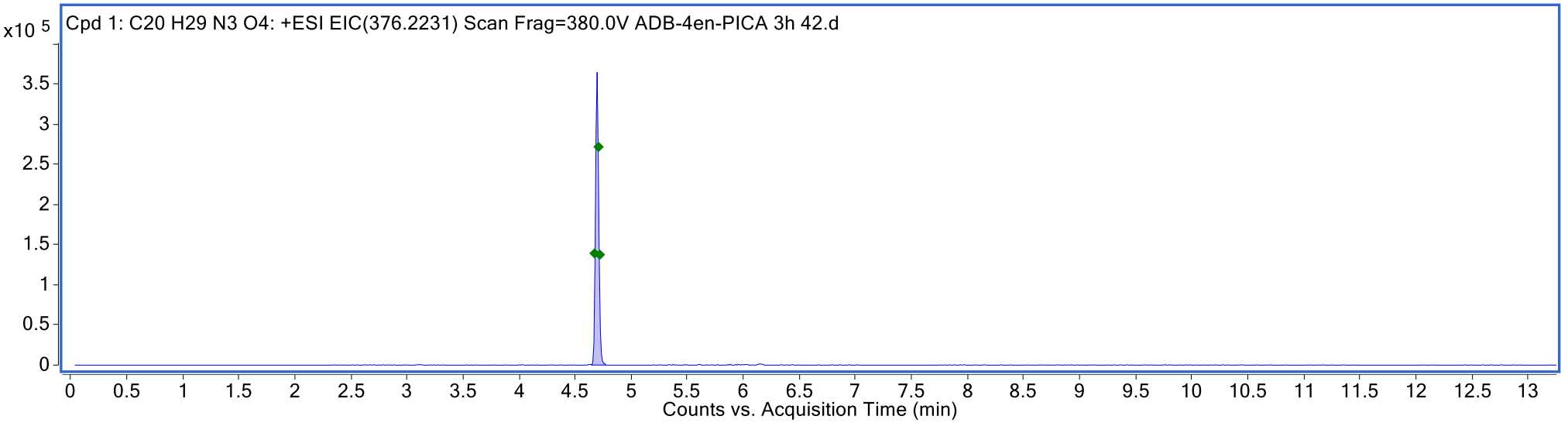


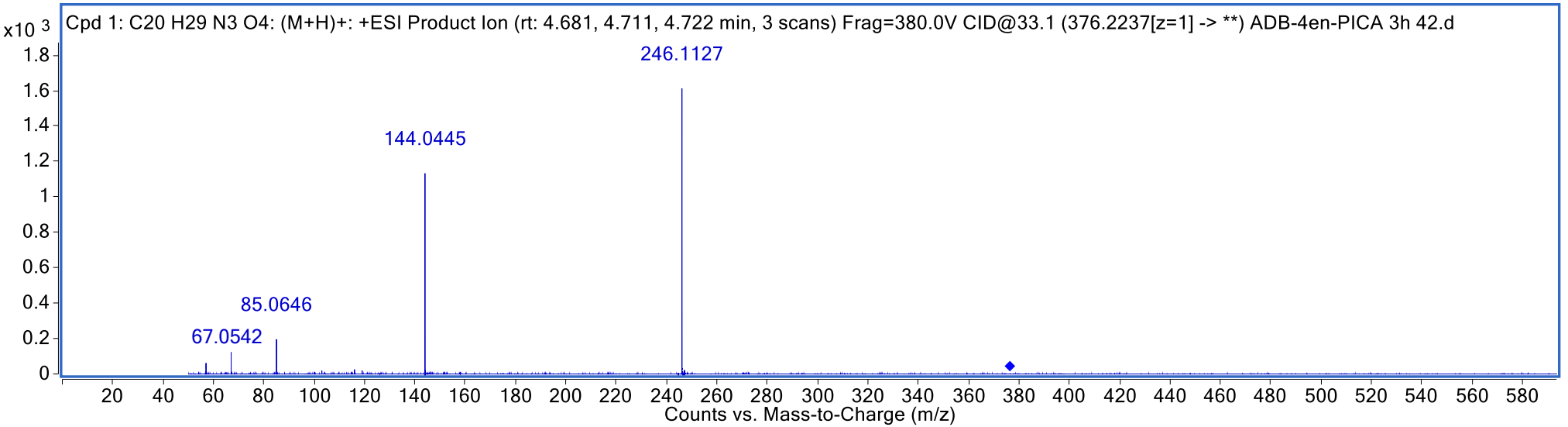
144.0444

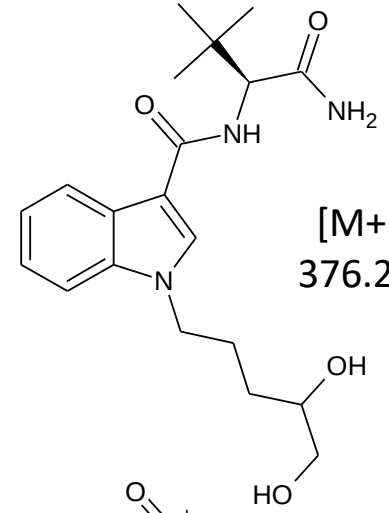


130.0651

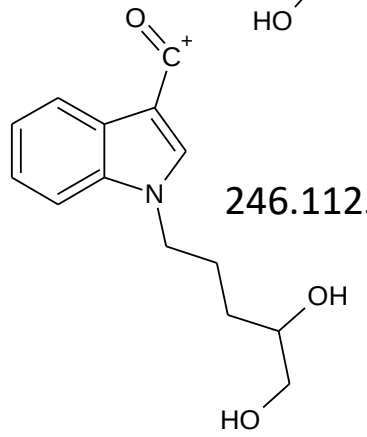
I2, Dihydrodiol formation, RT 4.69 min, *m/z* 376.2238



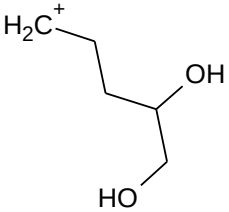




[M+H]+
376.2231

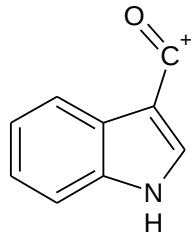


246.1125



103.0754
(not detected)

$-H_2O$

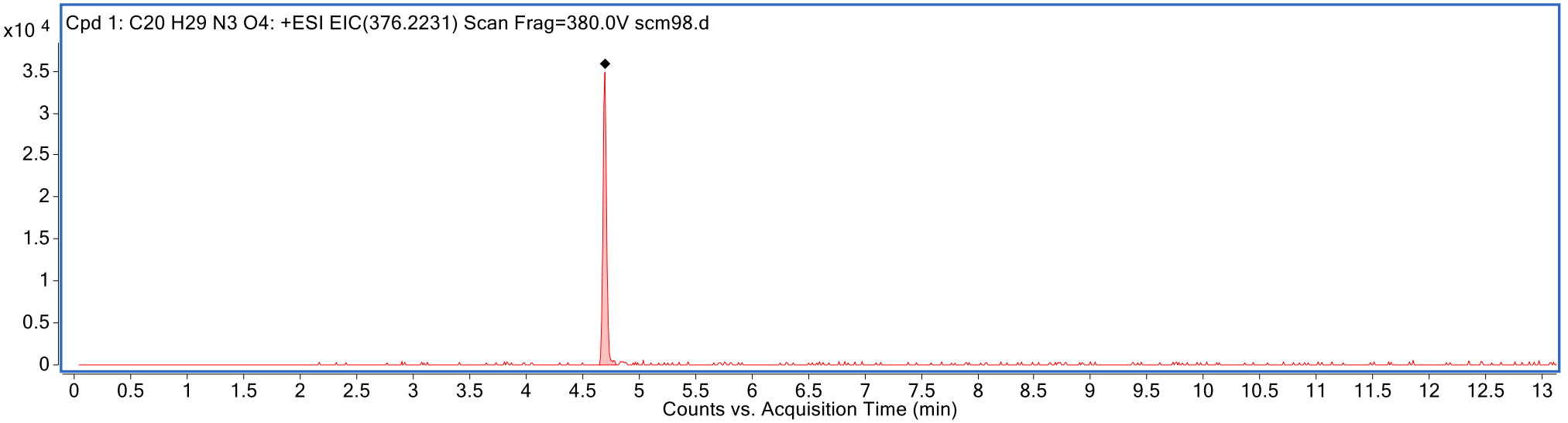


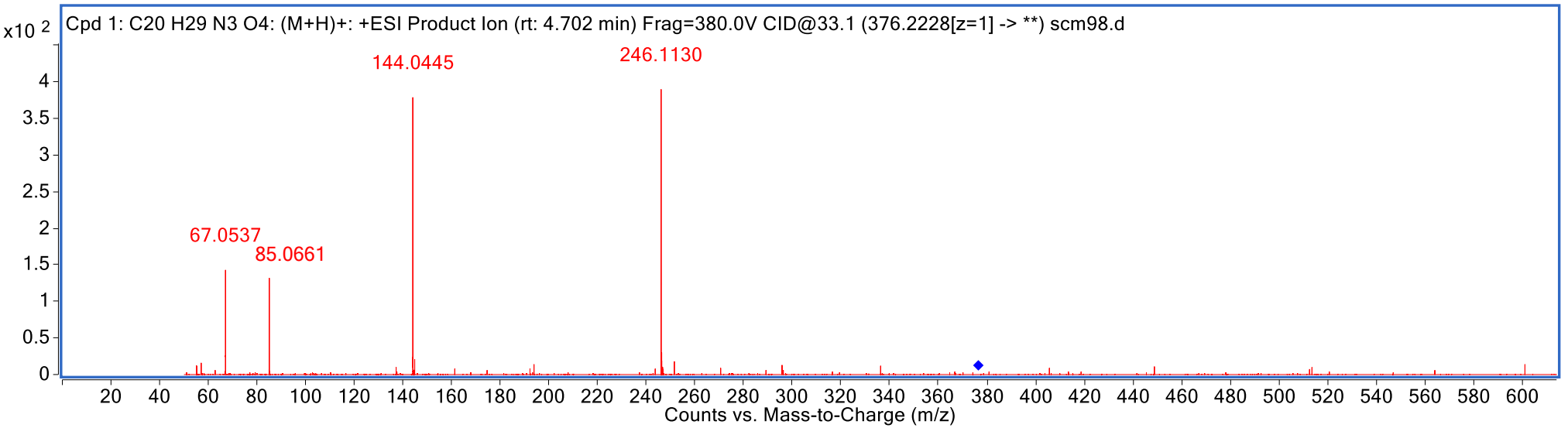
144.0444

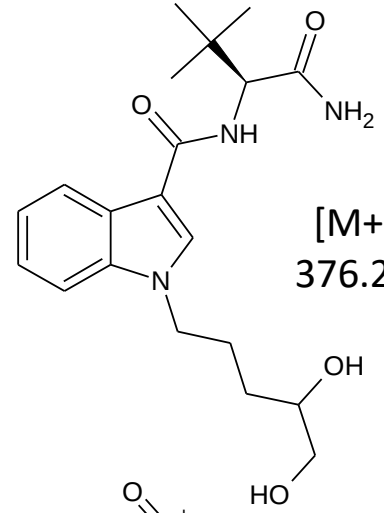
85.0648 → 67.0542

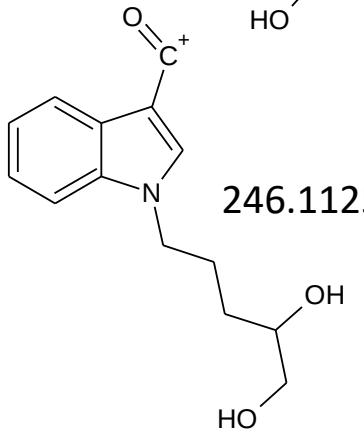
$-H_2O$

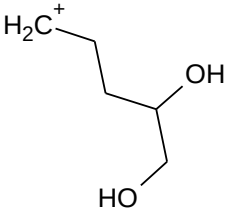
Dihydrodiol reference standard, RT 4.70 min, *m/z* 376.2228

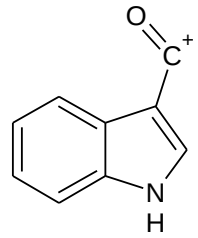




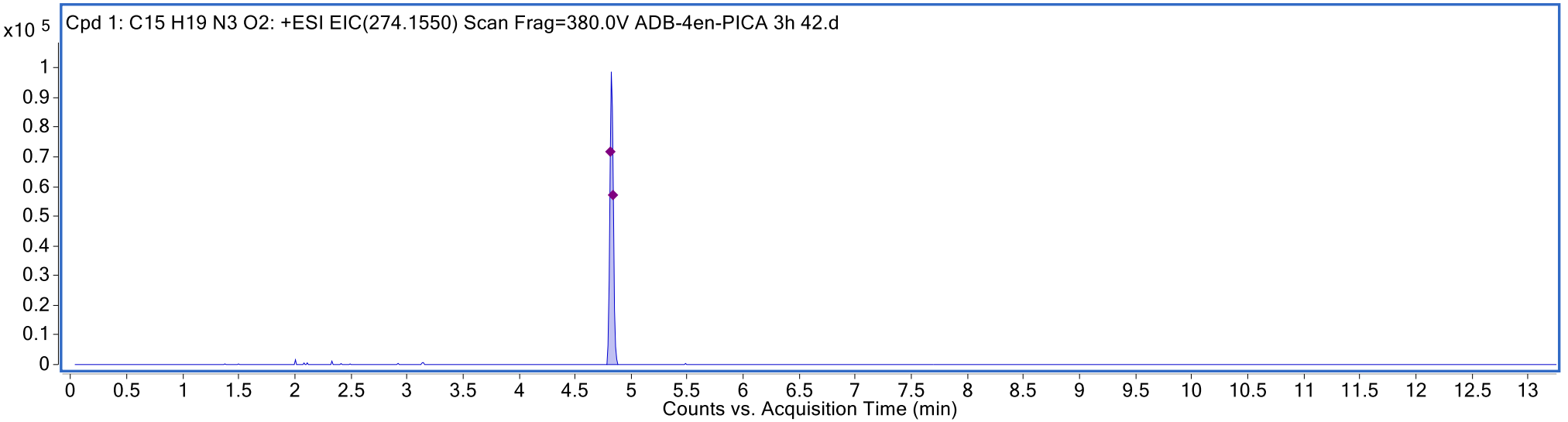


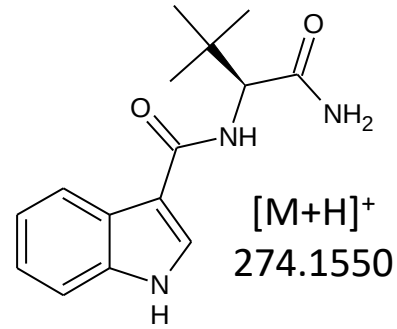




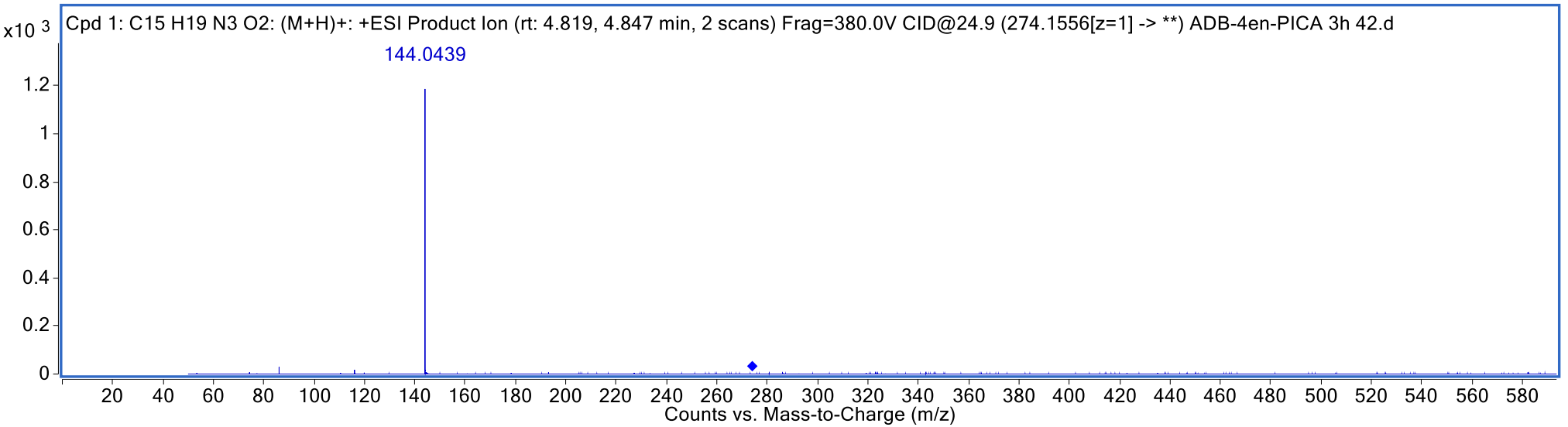


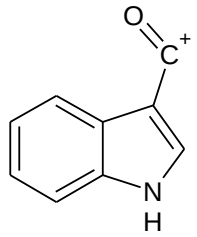
I3, *N*-dealkylation, RT 4.82 min, *m/z* 274.1555





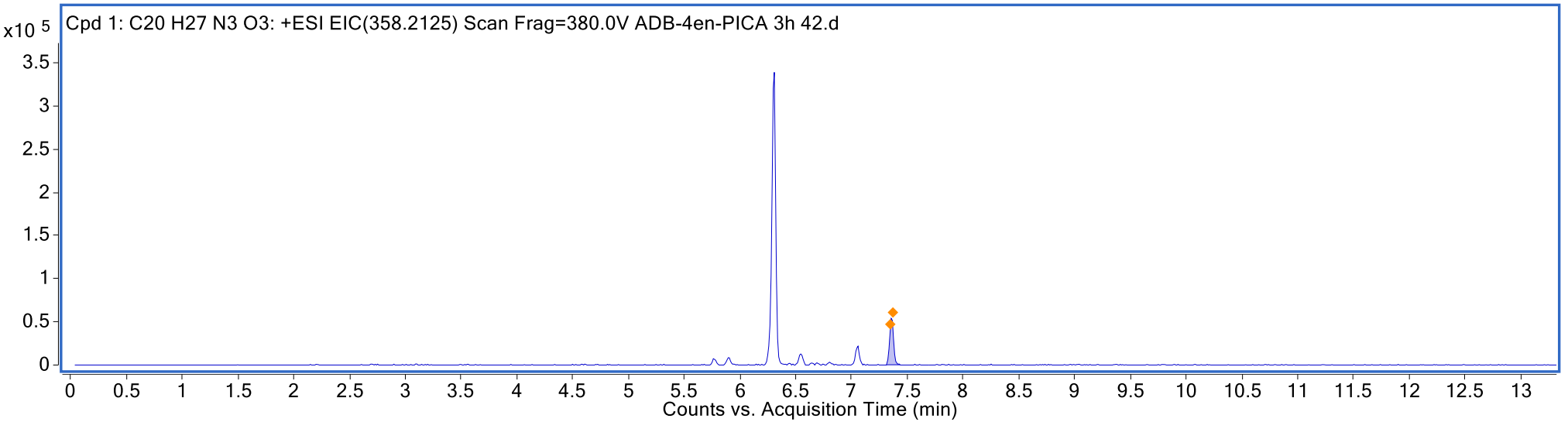
[M+H]+
274.1550

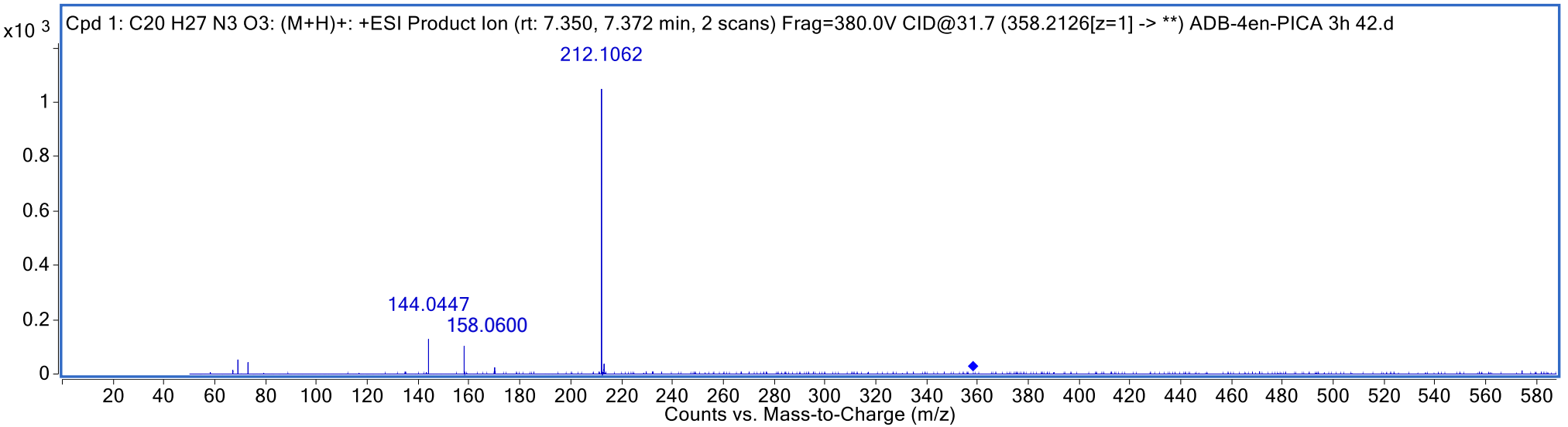


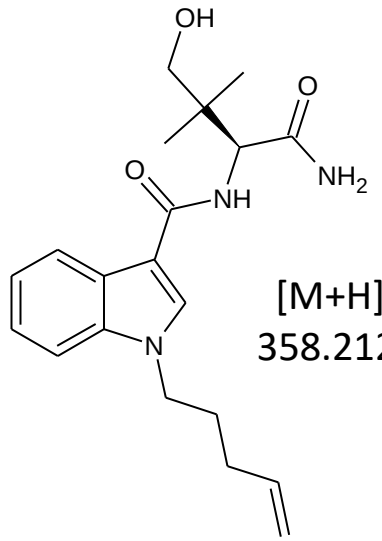


144.0444

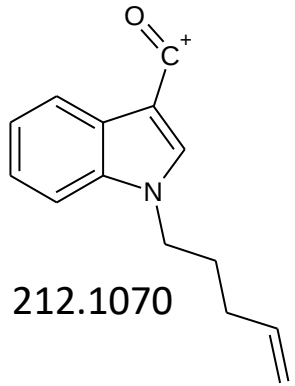
I4, Mono-hydroxylation (*tert*-butyl), RT 7.36 min, *m/z* 358.2131



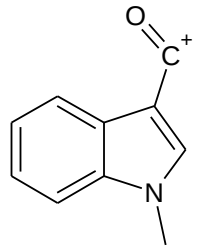




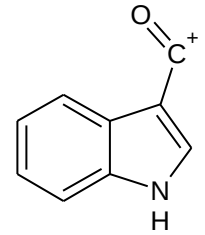
[M+H]+
358.2125



212.1070

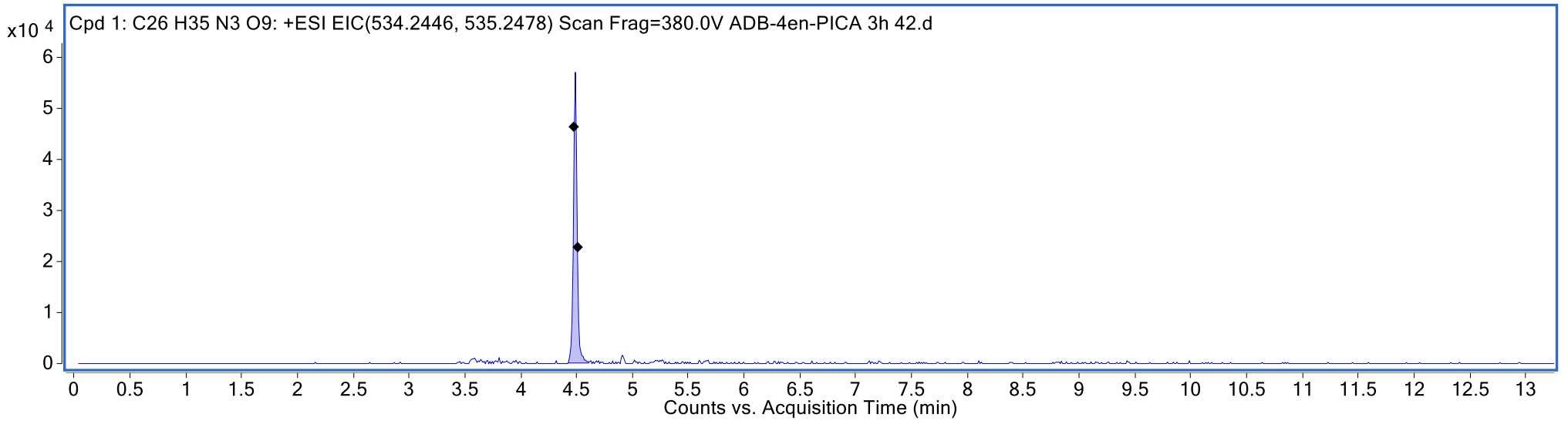


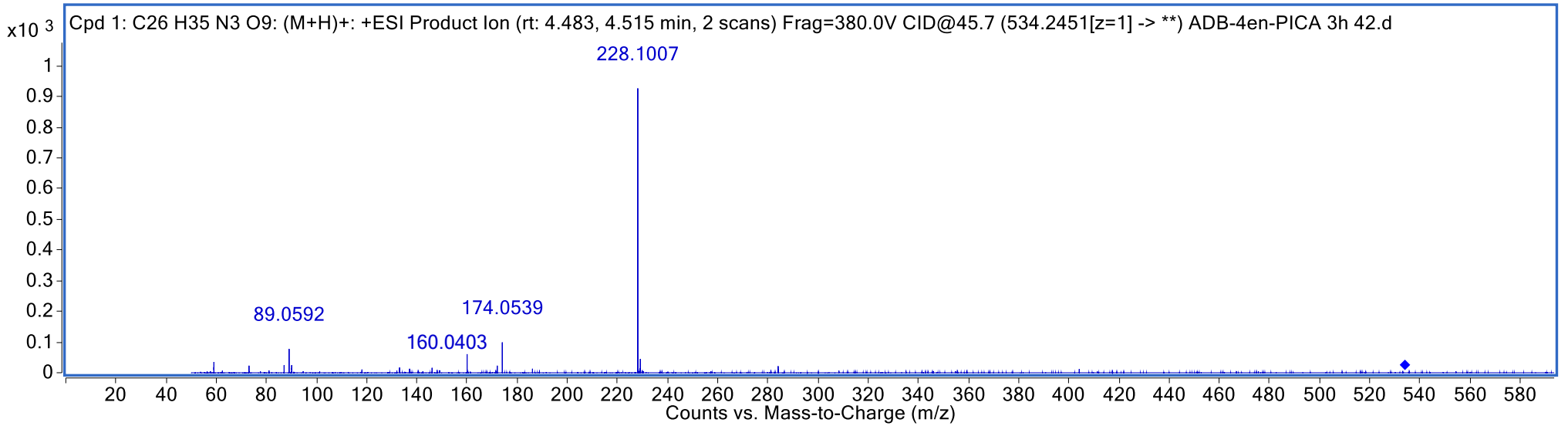
158.0600

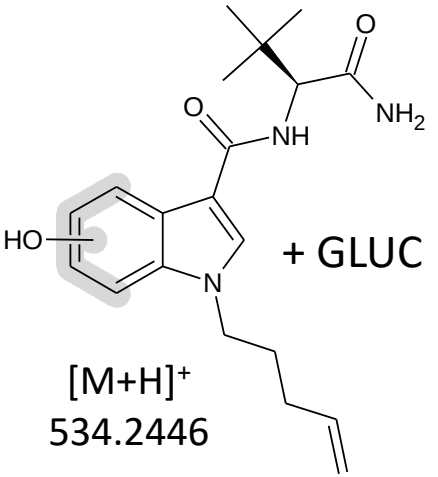


144.0444

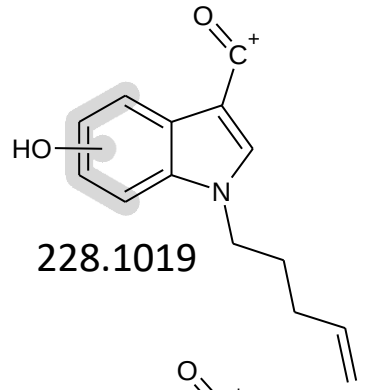
I5, Mono-hydroxylation (indole core) + glucuronidation, RT 4.49 min, *m/z* 534.2440



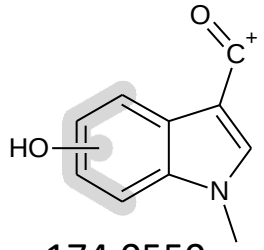




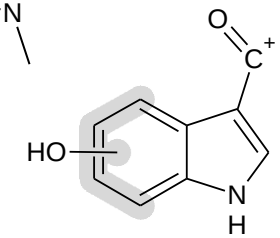
[M+H]+
534.2446



228.1019

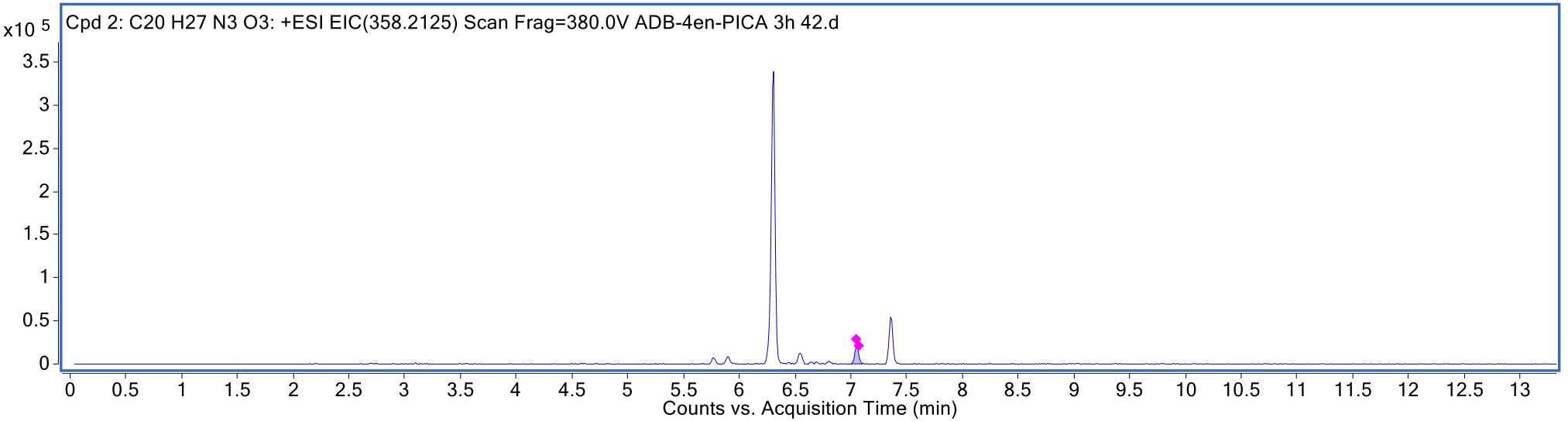


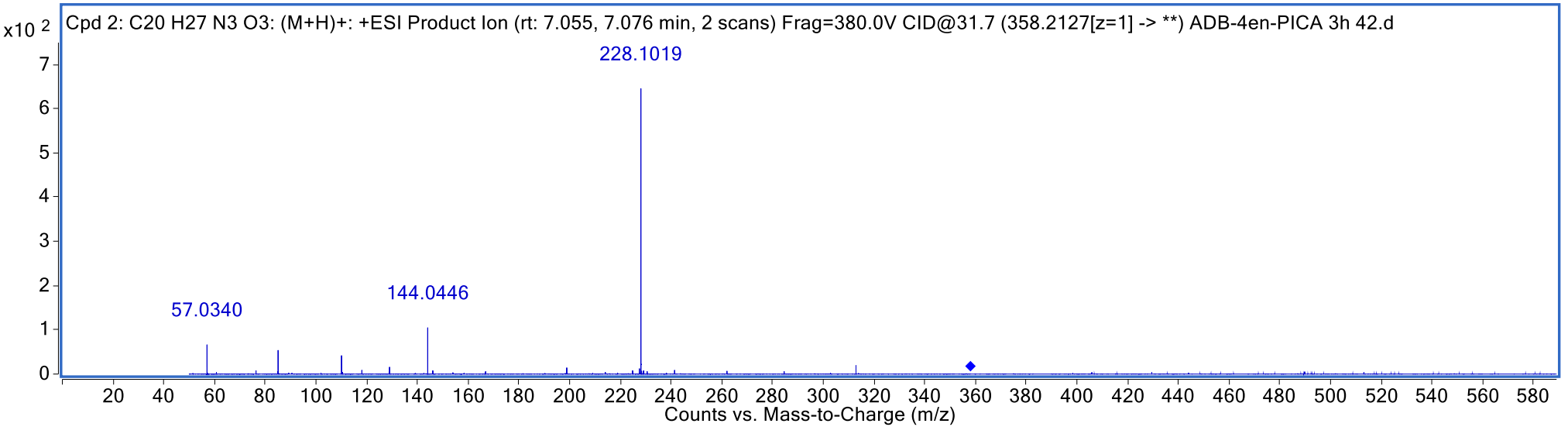
174.0550

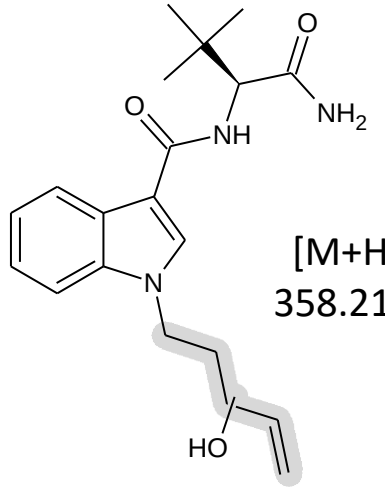


160.0393

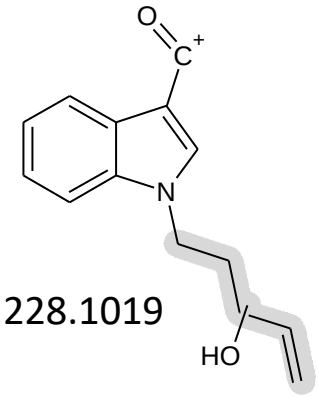
I6, Mono-hydroxylation (pentenyl tail), RT 7.05 min, *m/z* 358.2122



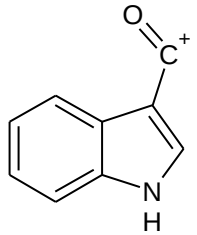




[M+H]+
358.2125

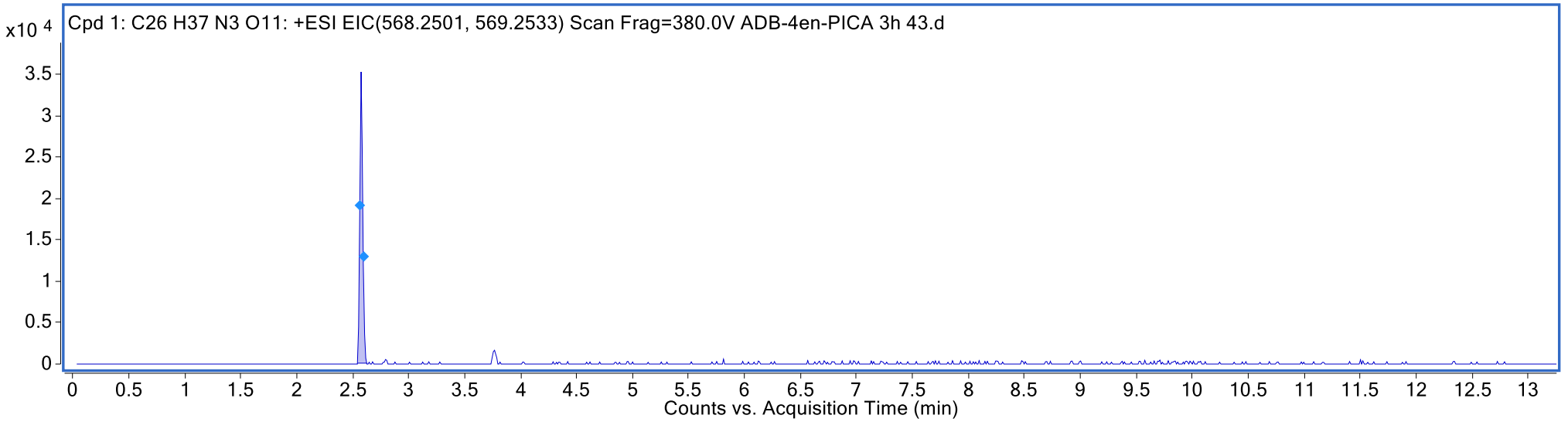


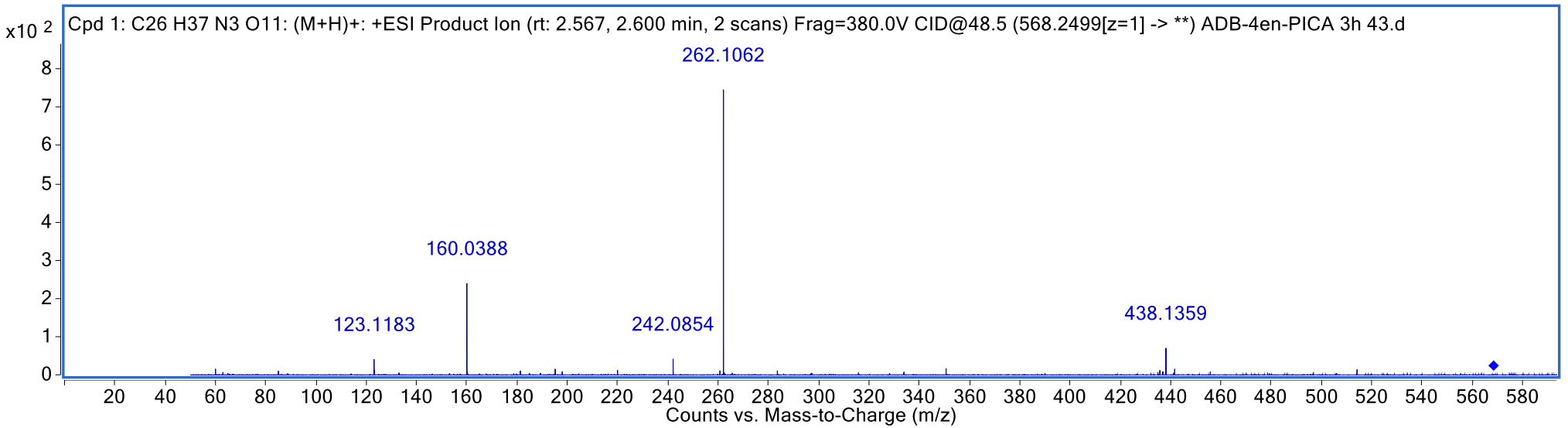
228.1019

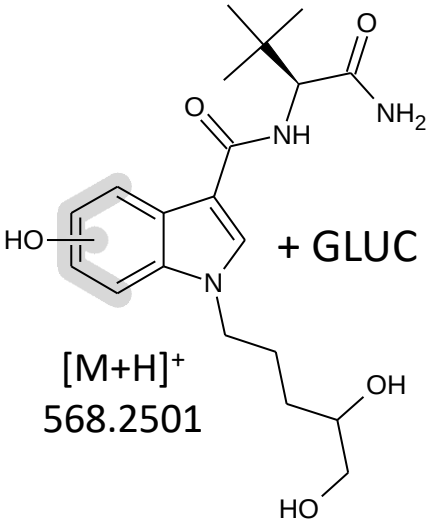


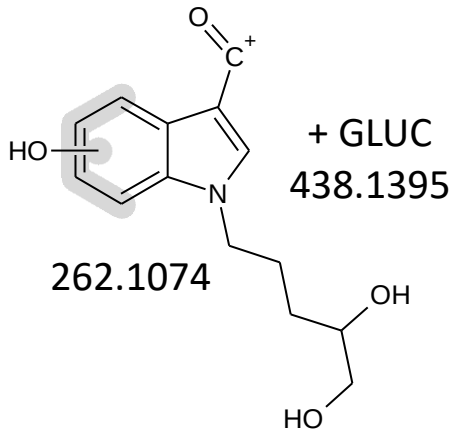
144.0444

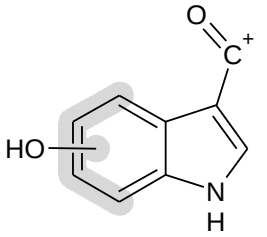
I7, Dihydrodiol formation + mono-hydroxylation (indole core) + glucuronidation, RT 2.58 min, *m/z* 568.2495



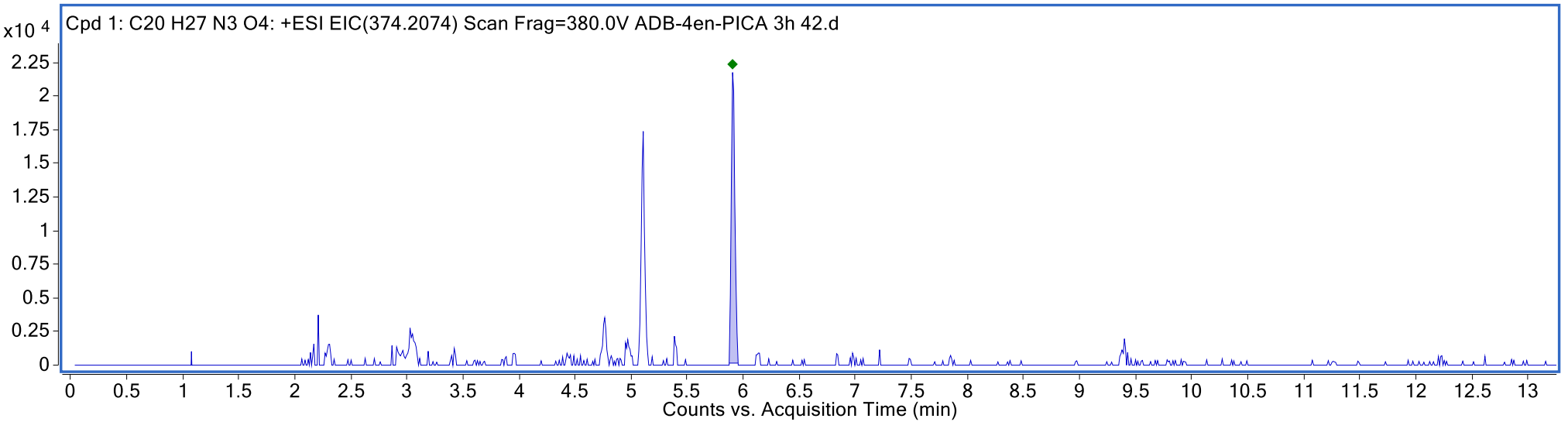


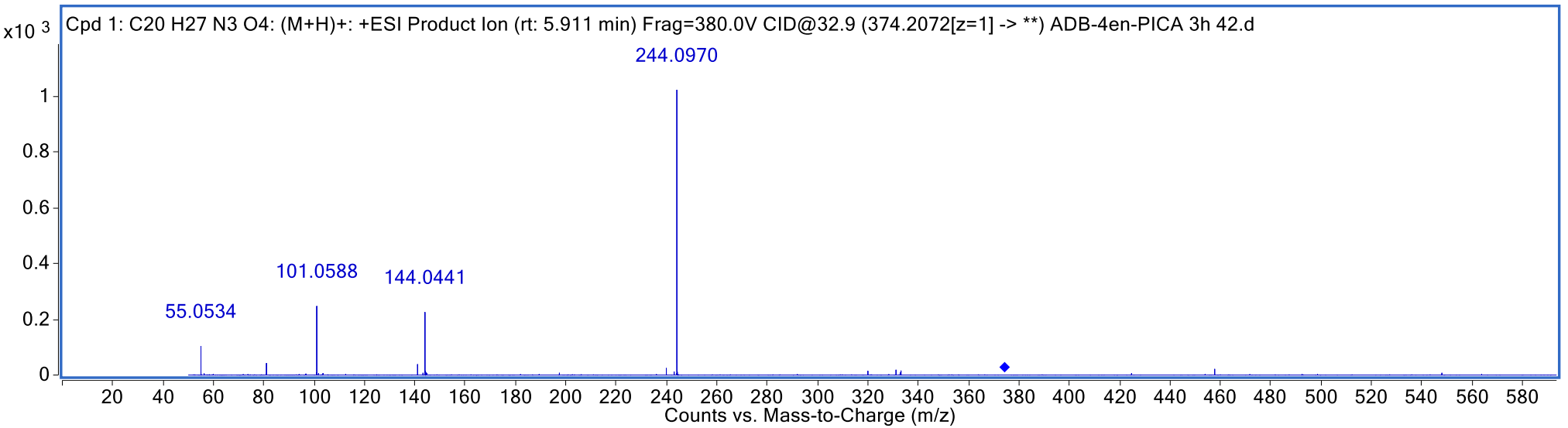


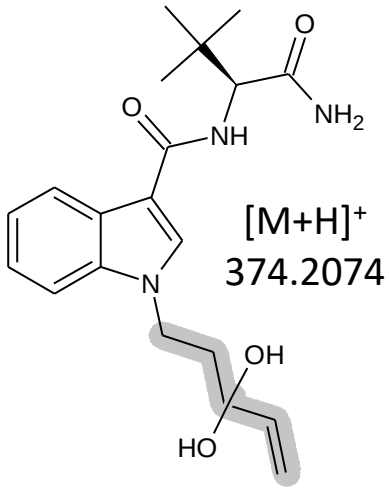


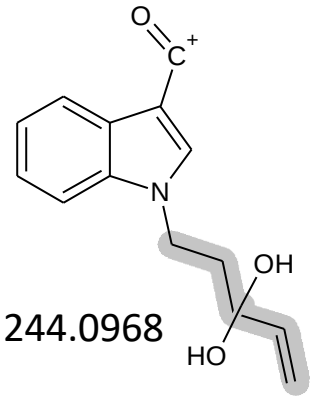


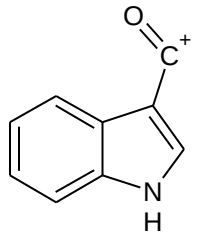
I8, Di-hydroxylation (pentenyl tail), RT 5.91 min, *m/z* 374.2073

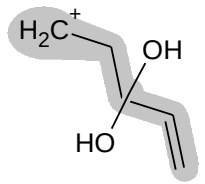




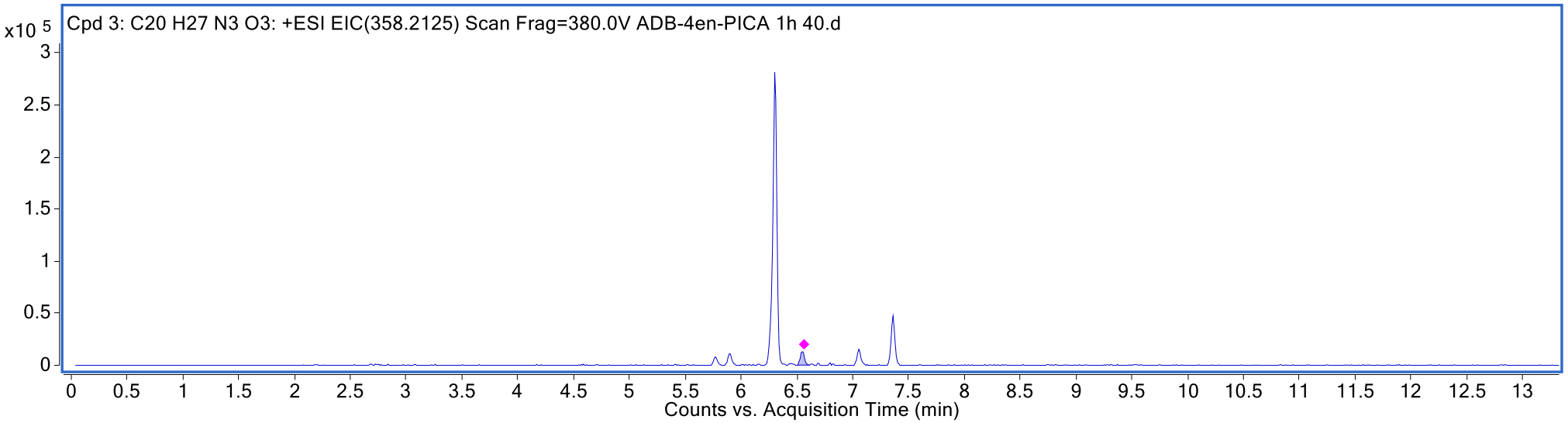


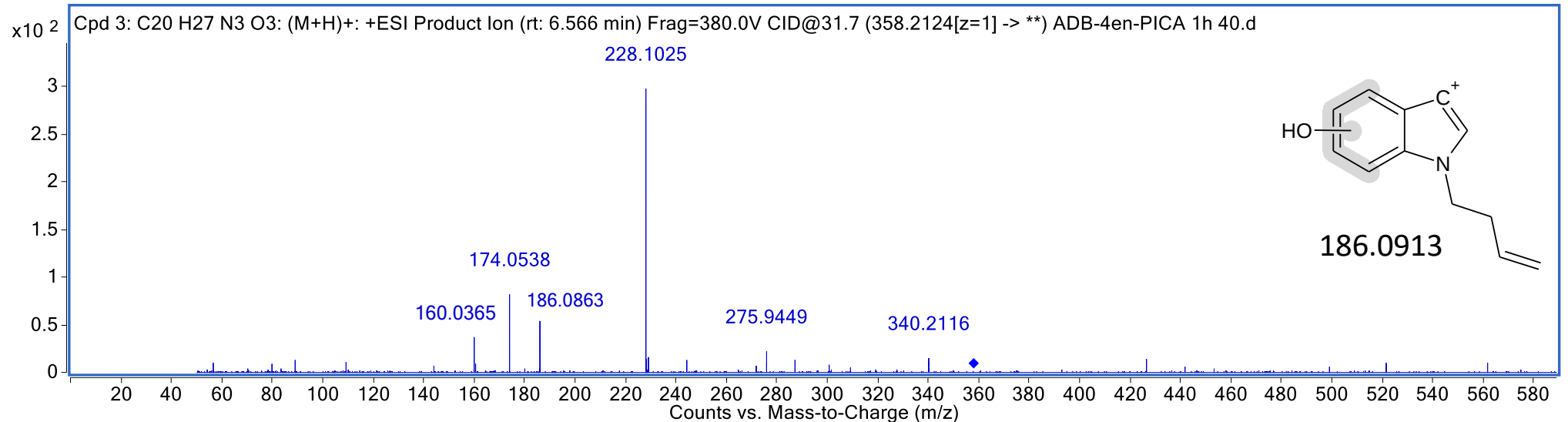


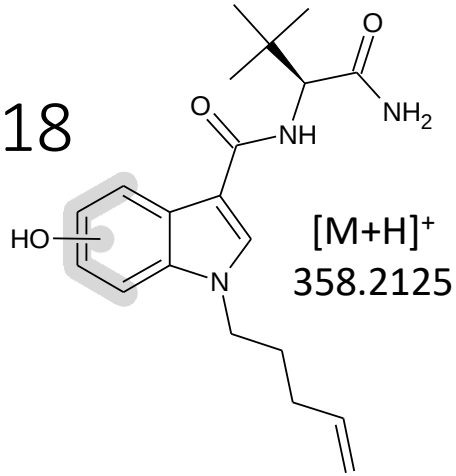


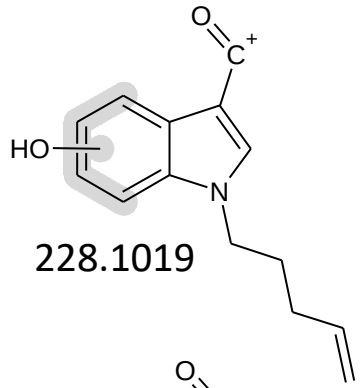


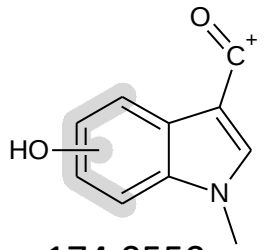
I9, Mono-hydroxylation (indole core), RT 6.54 min, *m/z* 358.2118

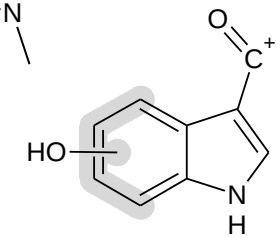




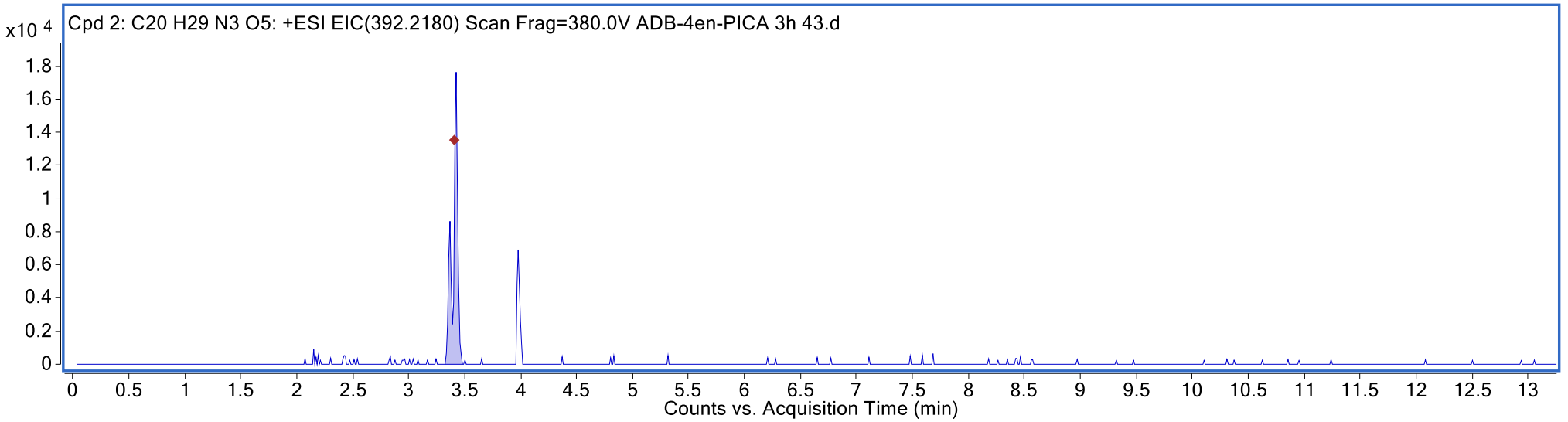


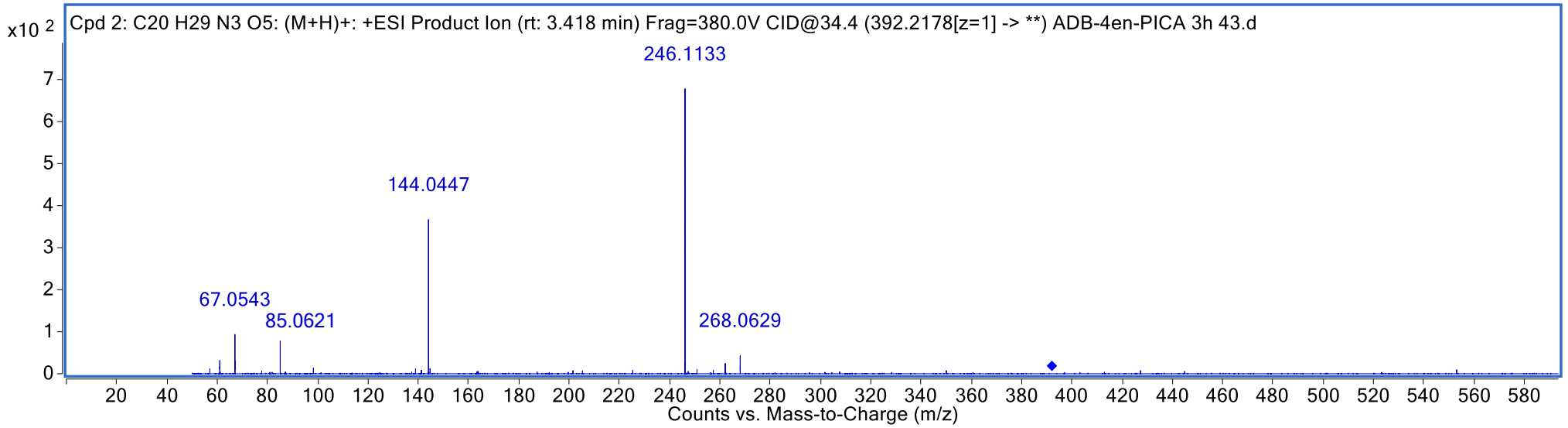


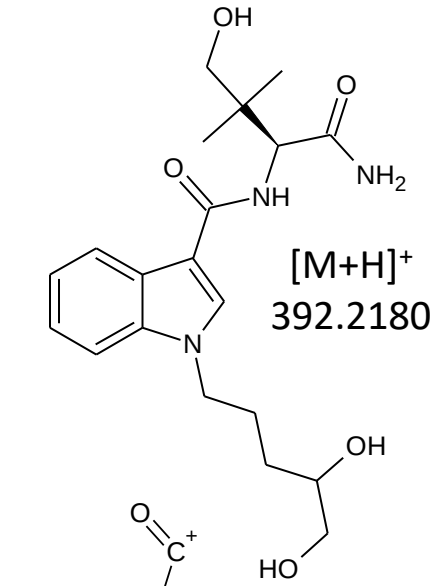


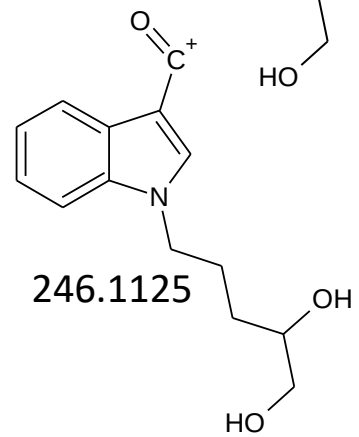


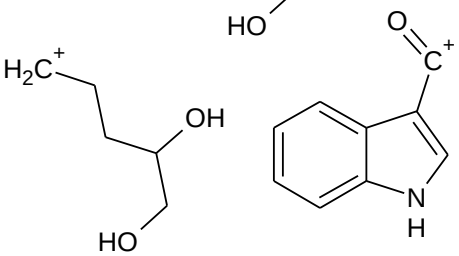
I10, Dihydrodiol formation + mono-hydroxylation (*tert*-butyl), RT 3.42 min, *m/z* 392.2178







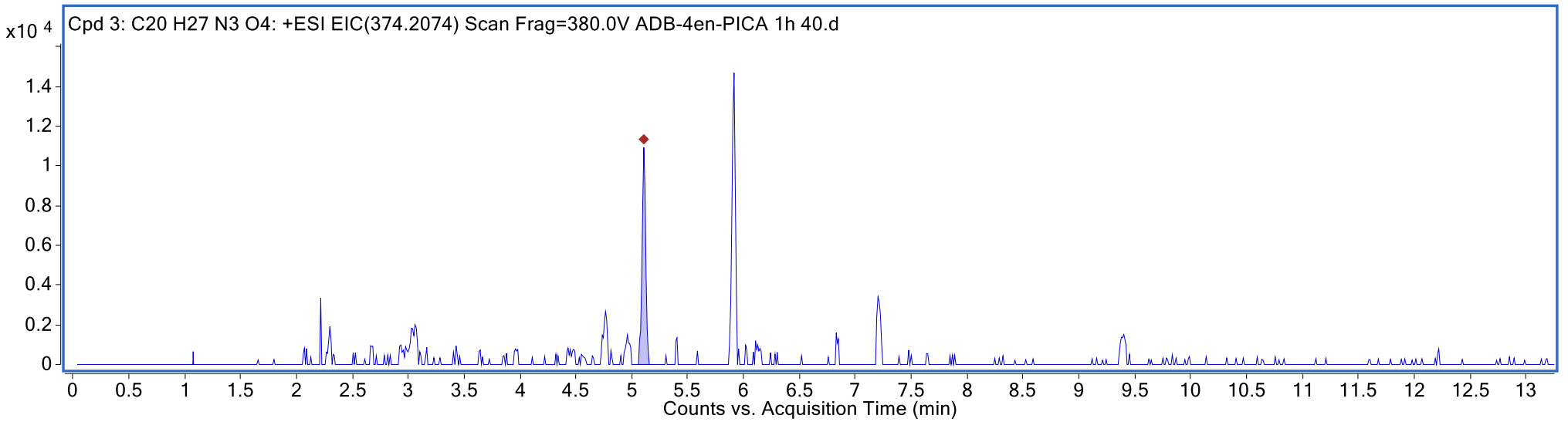


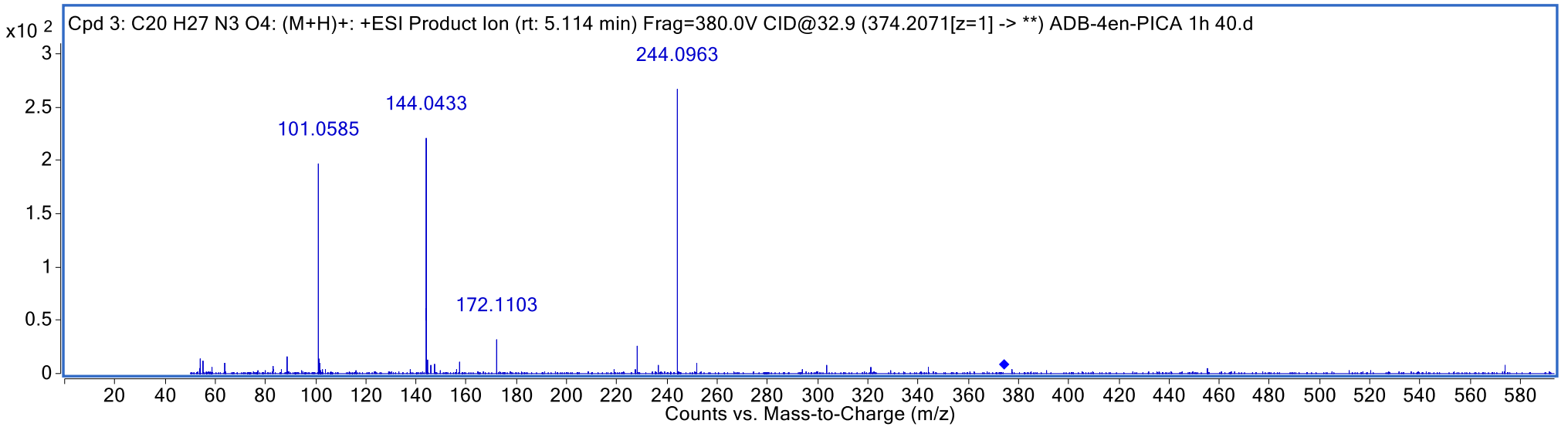


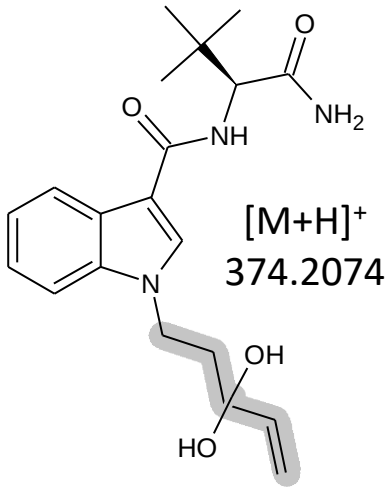
$-H_2O$

85.0648 → 67.0542 ($-H_2O$)

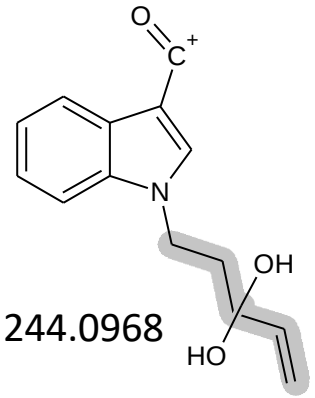
I11, Di-hydroxylation (pentenyl tail), RT 5.10 min, *m/z* 374.2069



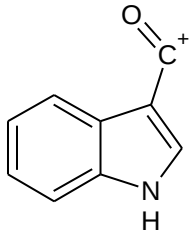




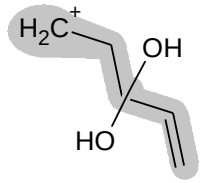
[M+H]+
374.2074



244.0968

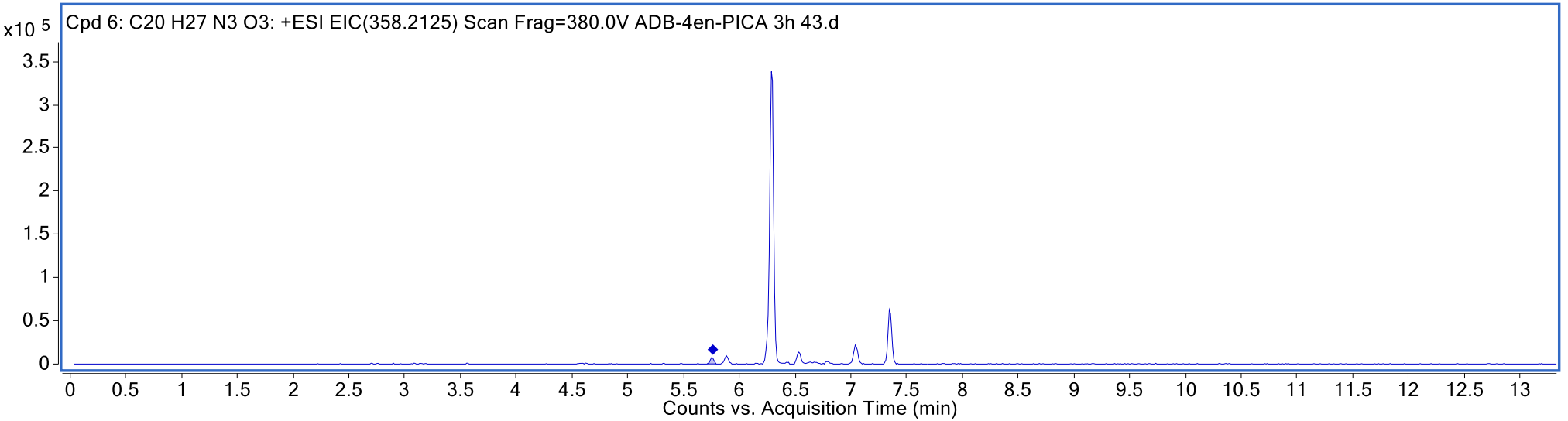


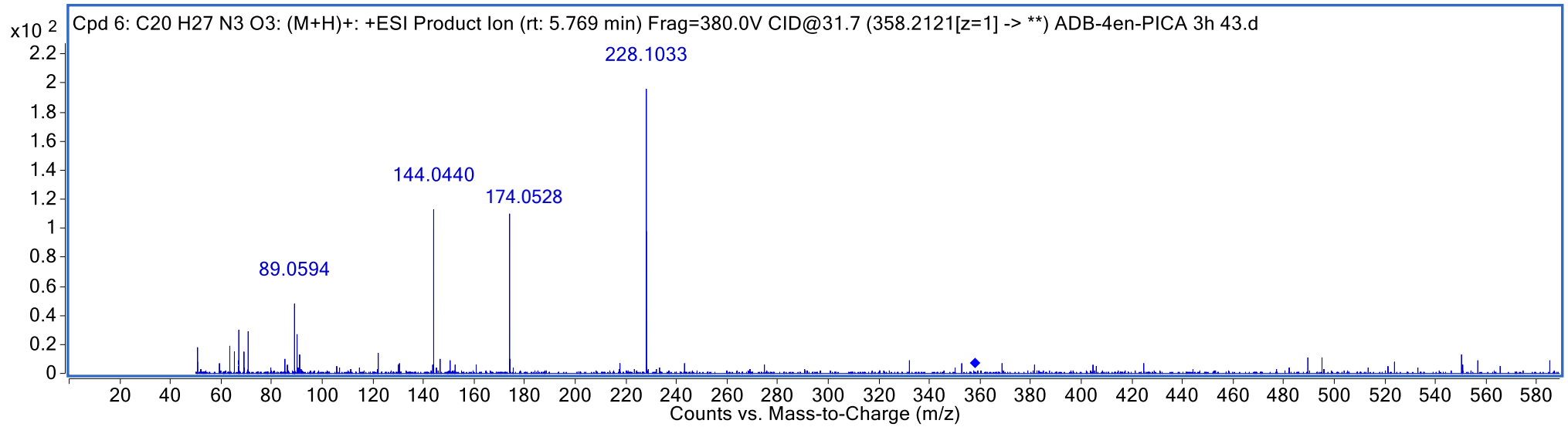
144.0444

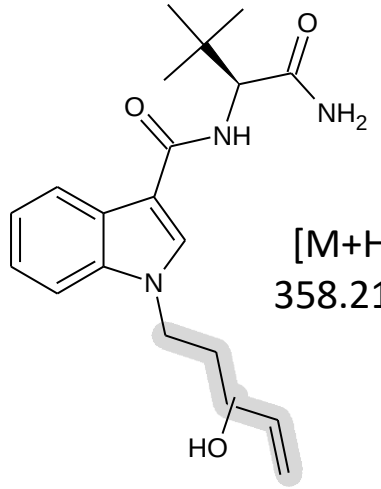


101.0597

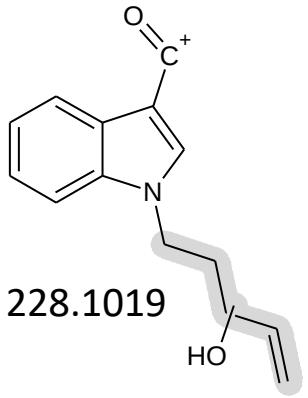
I12, Mono-hydroxylation (pentenyl tail), RT 5.76 min, *m/z* 358.2121



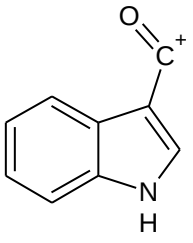




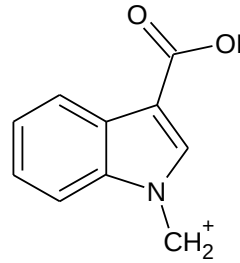
$[M+H]^+$
358.2125



228.1019



144.0444

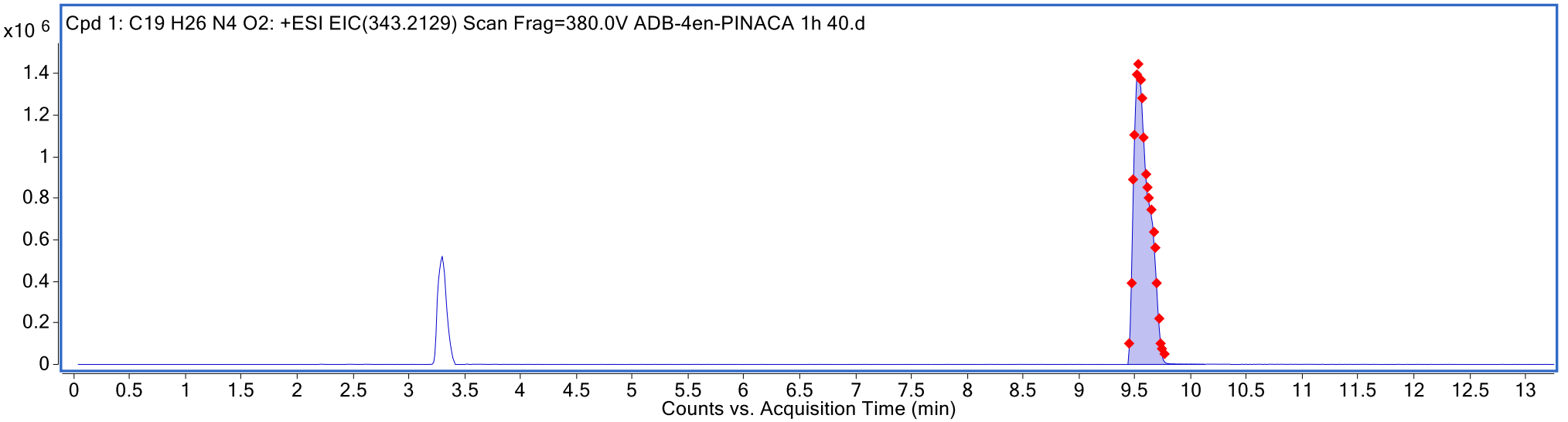


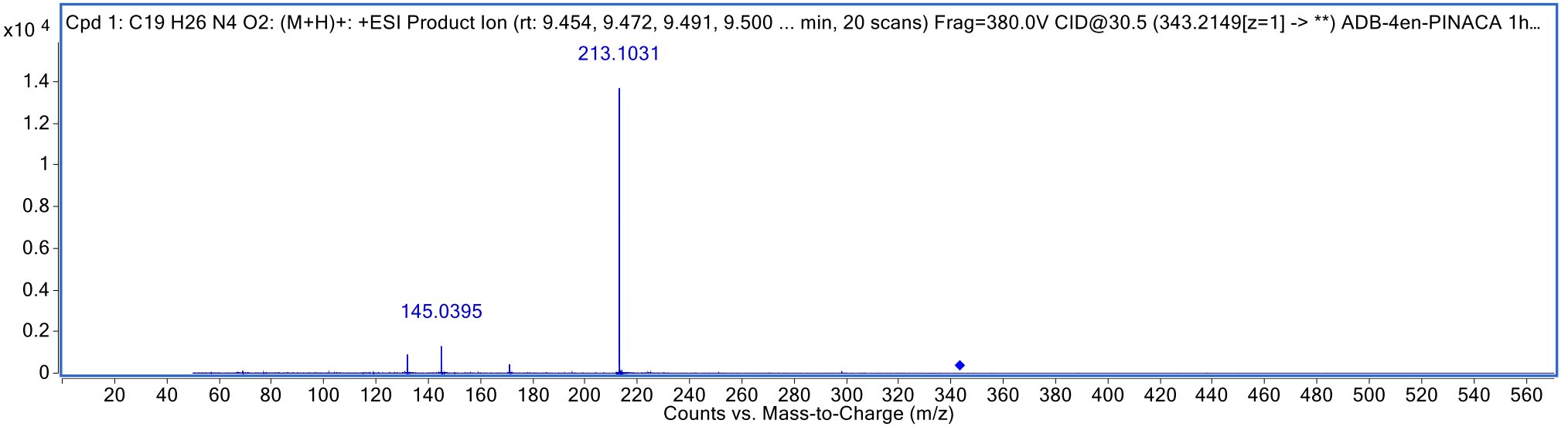
174.0550

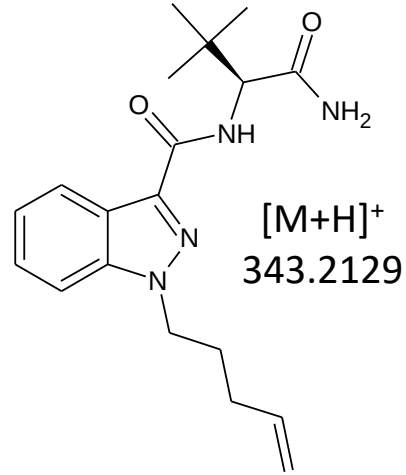
ADB-4en-PINACA

Metabolism

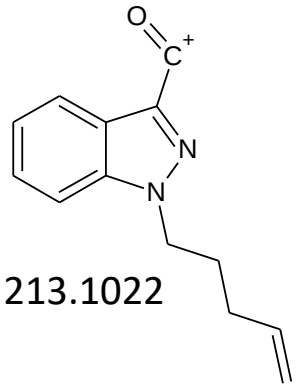
ADB-4en-PINACA, RT 9.54 min, *m/z* 343.2150



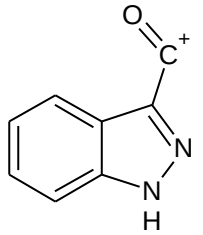




[M+H]+
343.2129

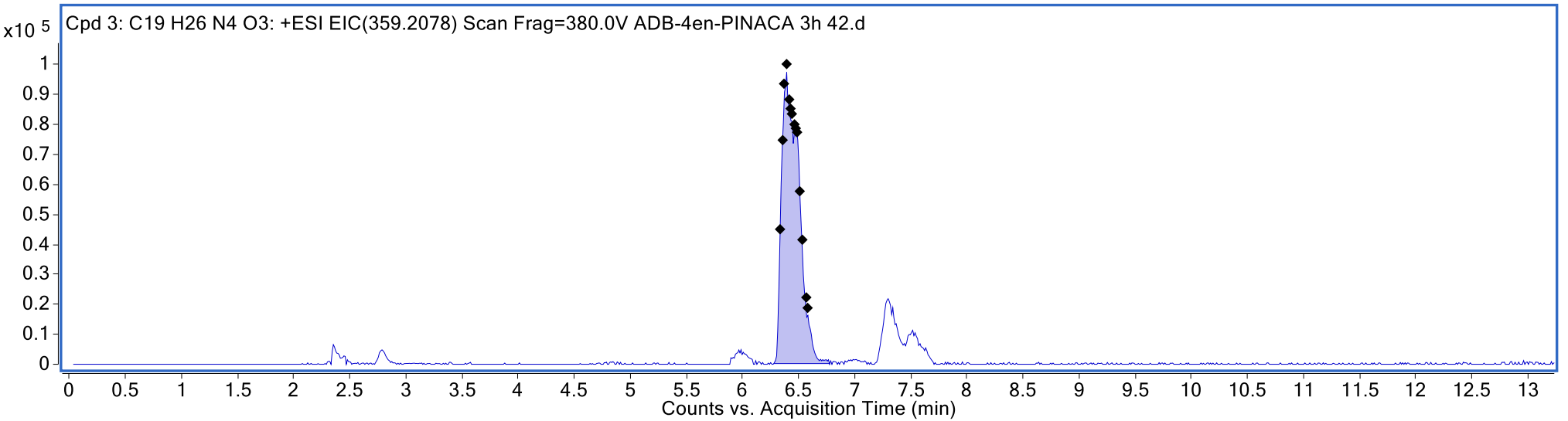


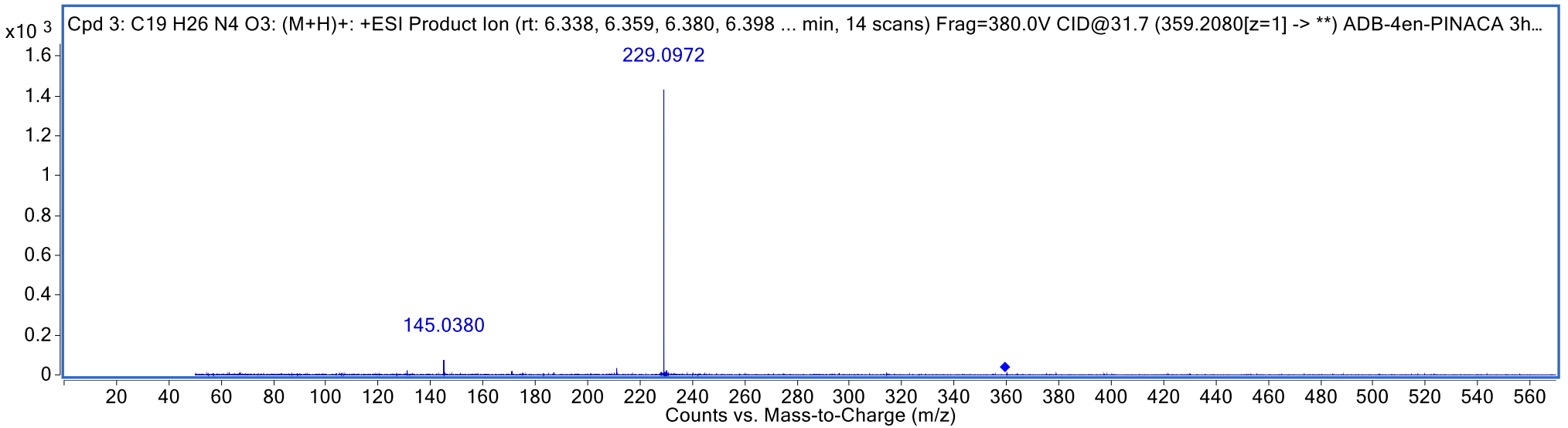
213.1022

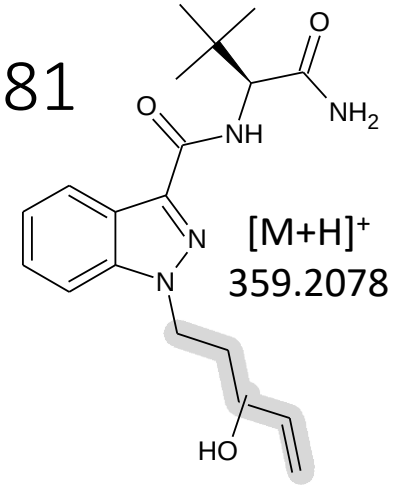


145.0396

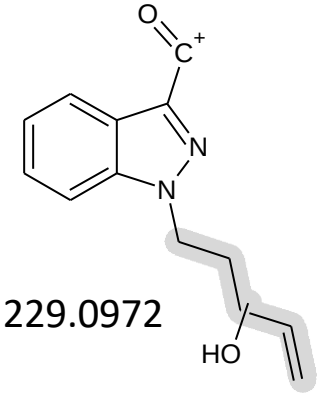
J1, Mono-hydroxylation (pentenyl tail), RT 6.41 min, *m/z* 359.2081



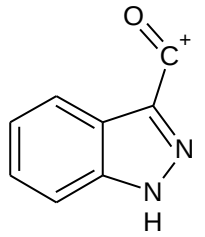




[M+H]+
359.2078

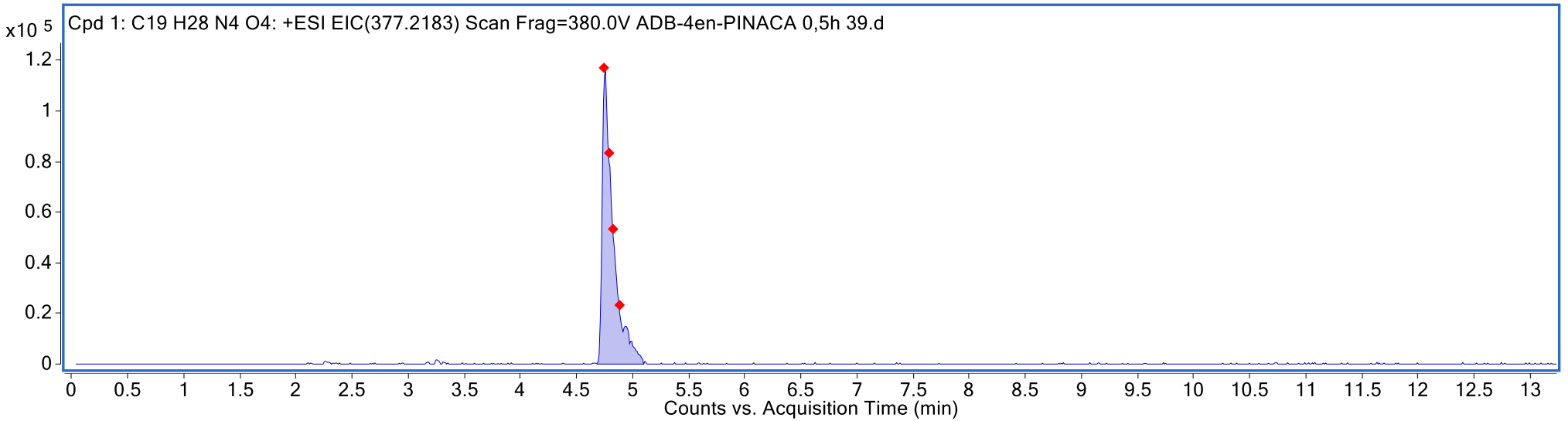


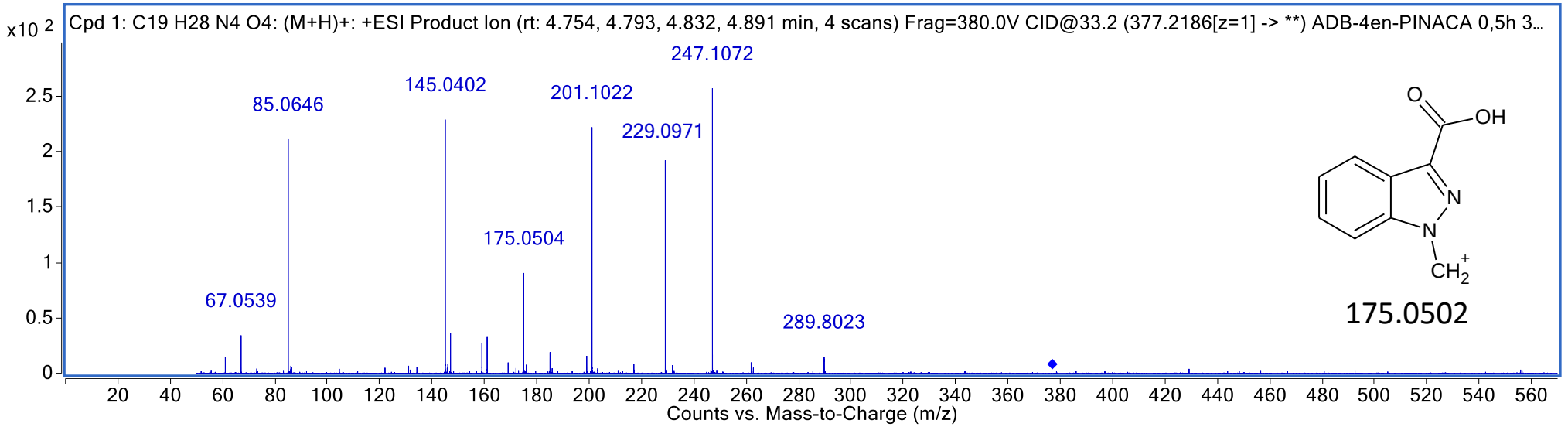
229.0972

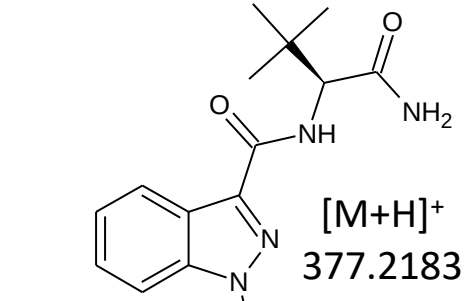


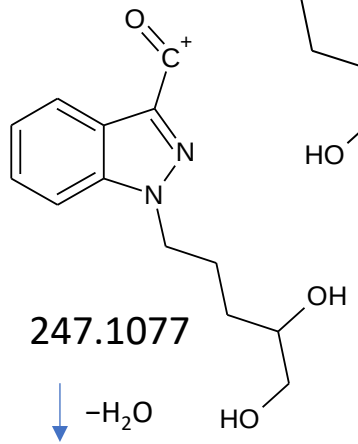
145.0396

J2, Dihydrodiol formation, RT 4.76 min, *m/z* 377.2184

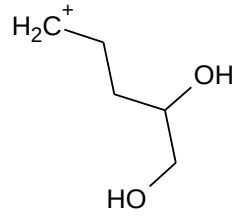


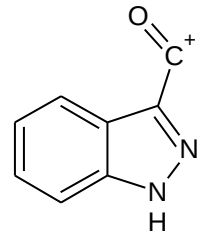






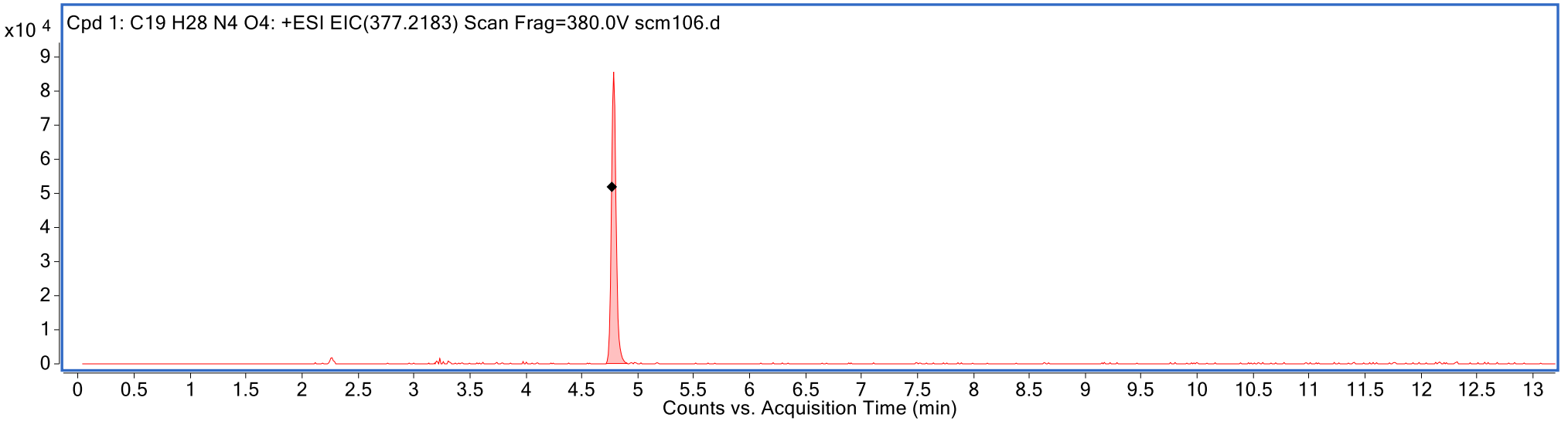
229.0972 → 201.1022 (−CO)

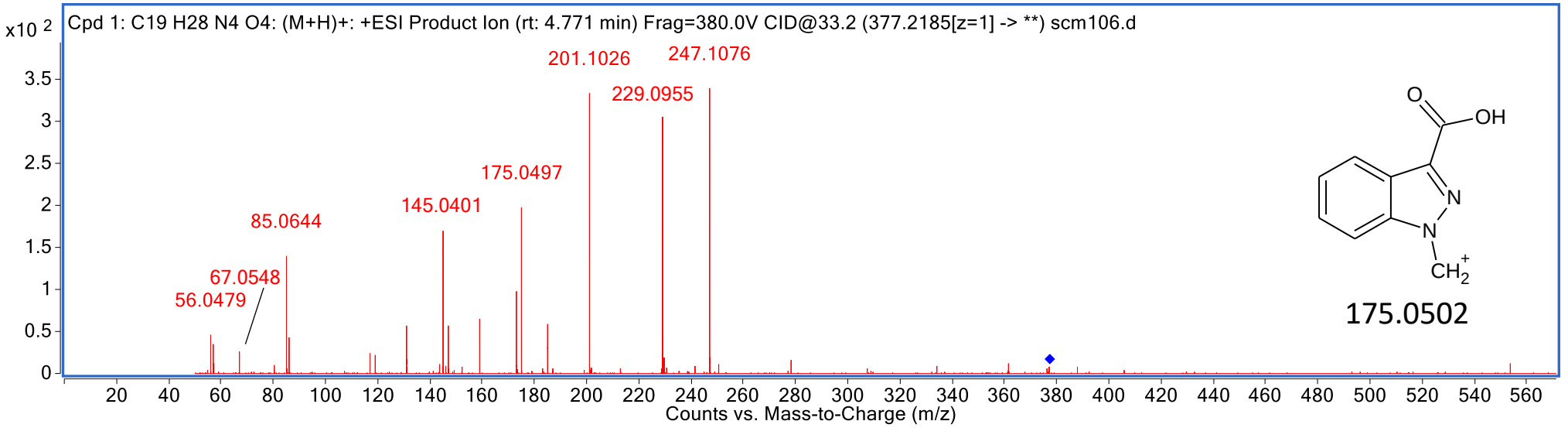


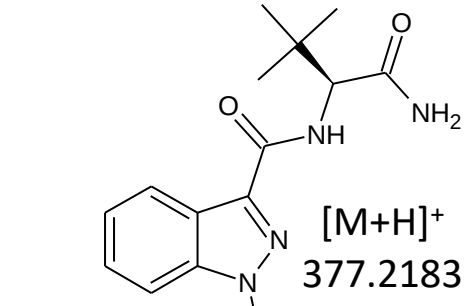


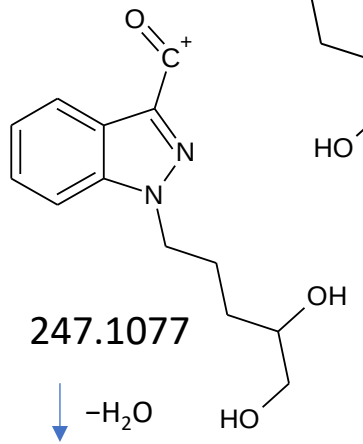
85.0648 → 67.0542 (−H2O)

Dihydrodiol reference standard, RT 4.78 min, *m/z* 377.2185

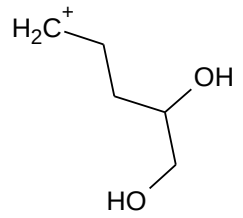


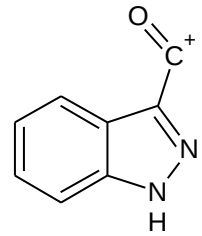






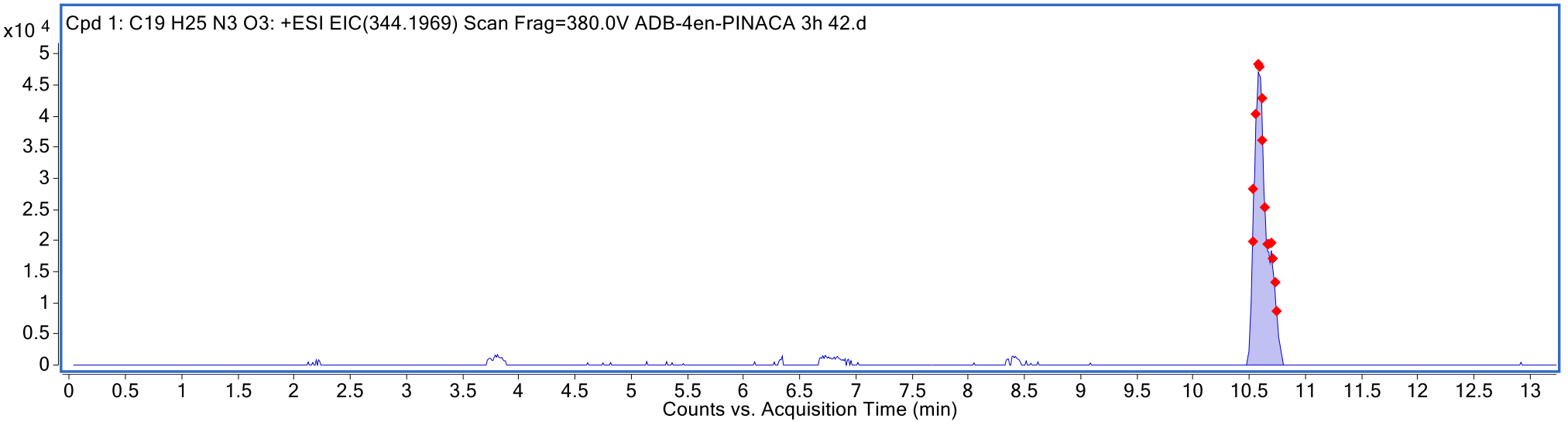
229.0972 → 201.1022 (−CO)

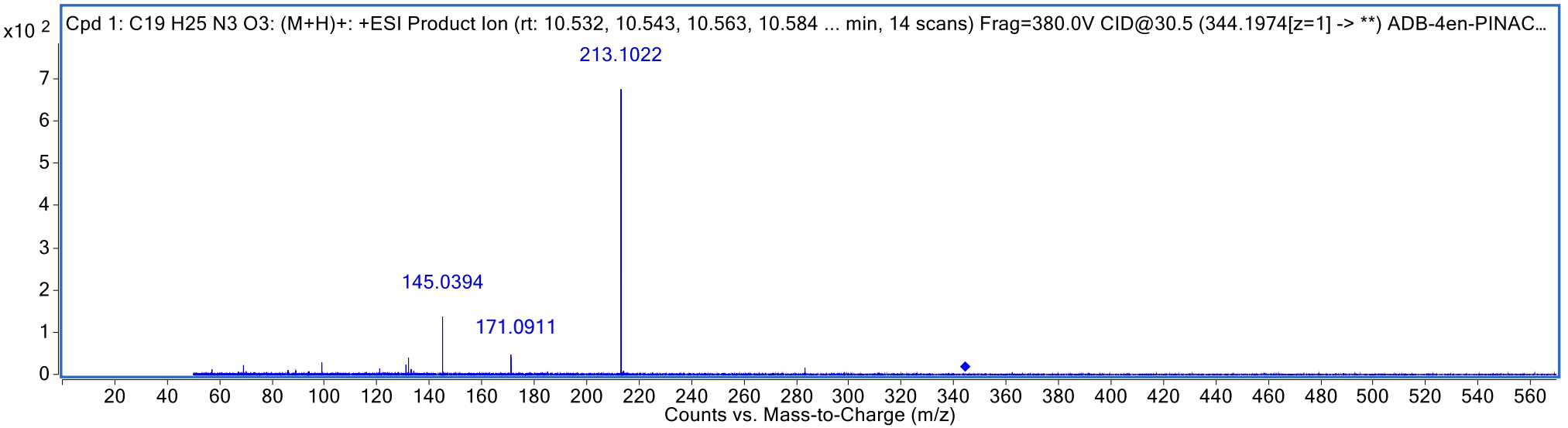


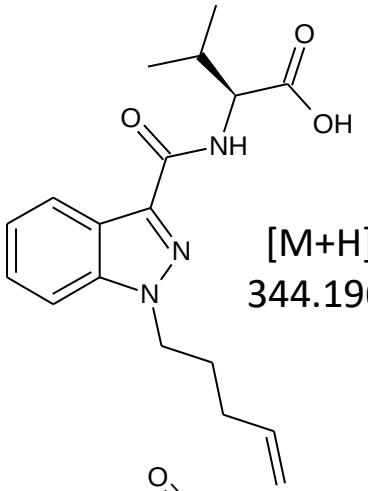


85.0648 → 67.0542 (−H2O)

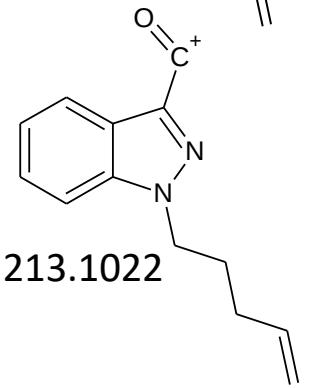
J3, Terminal amide hydrolysis, RT 10.58 min, *m/z* 344.1966



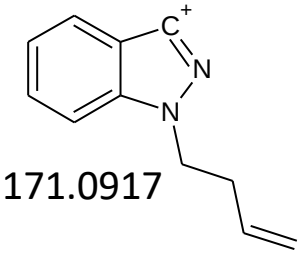




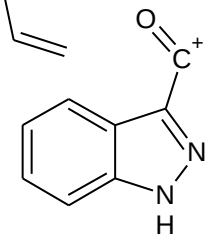
$[M+H]^+$
344.1969



213.1022

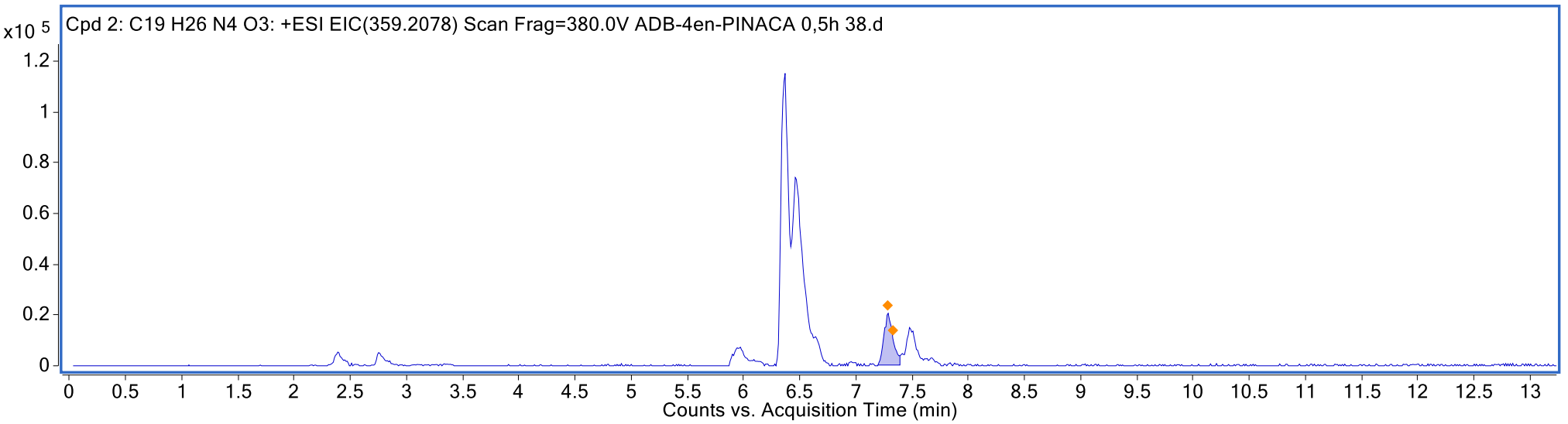


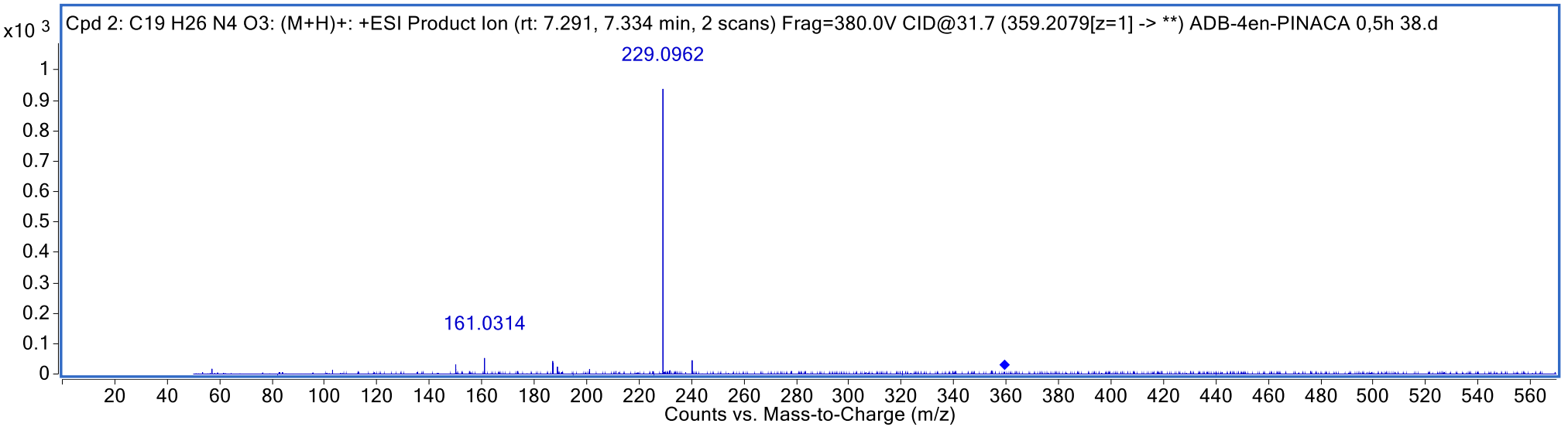
171.0917

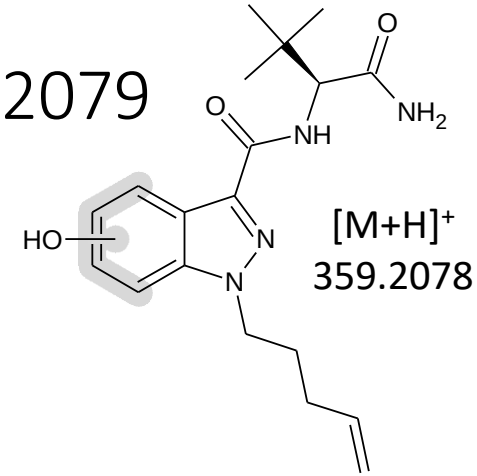


145.0396

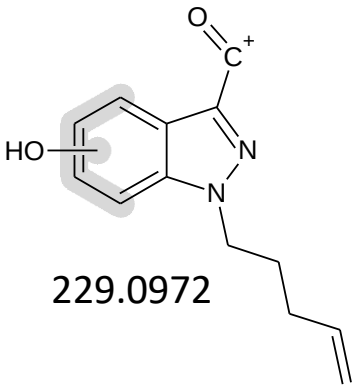
J4, Mono-hydroxylation (indazole core), RT 7.30 min, *m/z* 359.2079



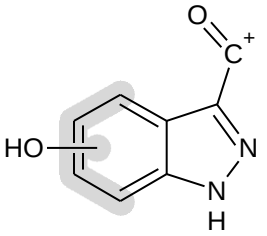




[M+H]+
359.2078

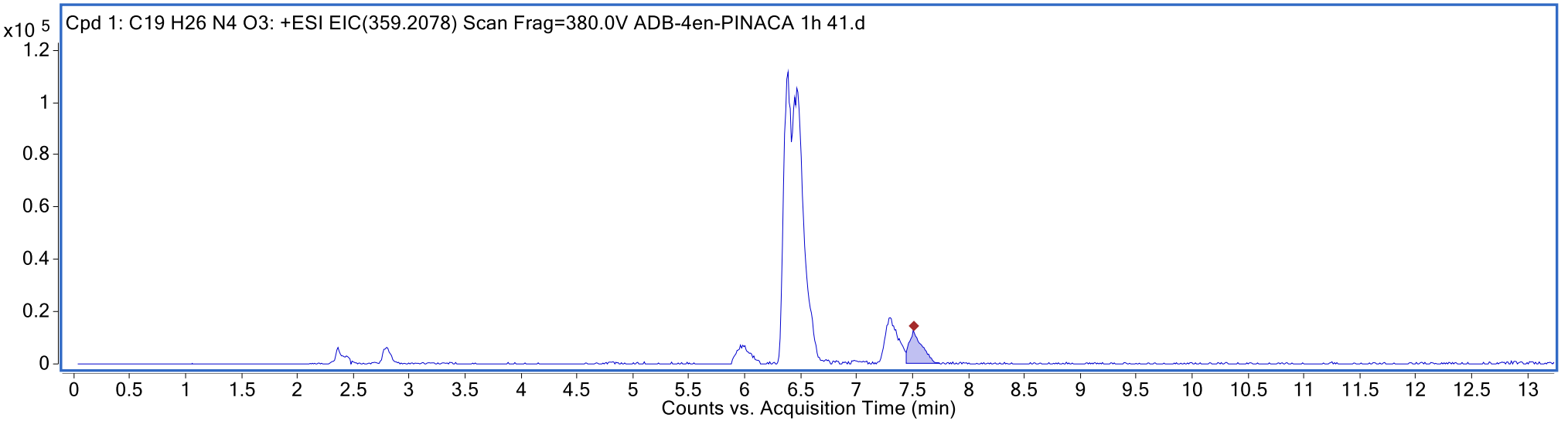


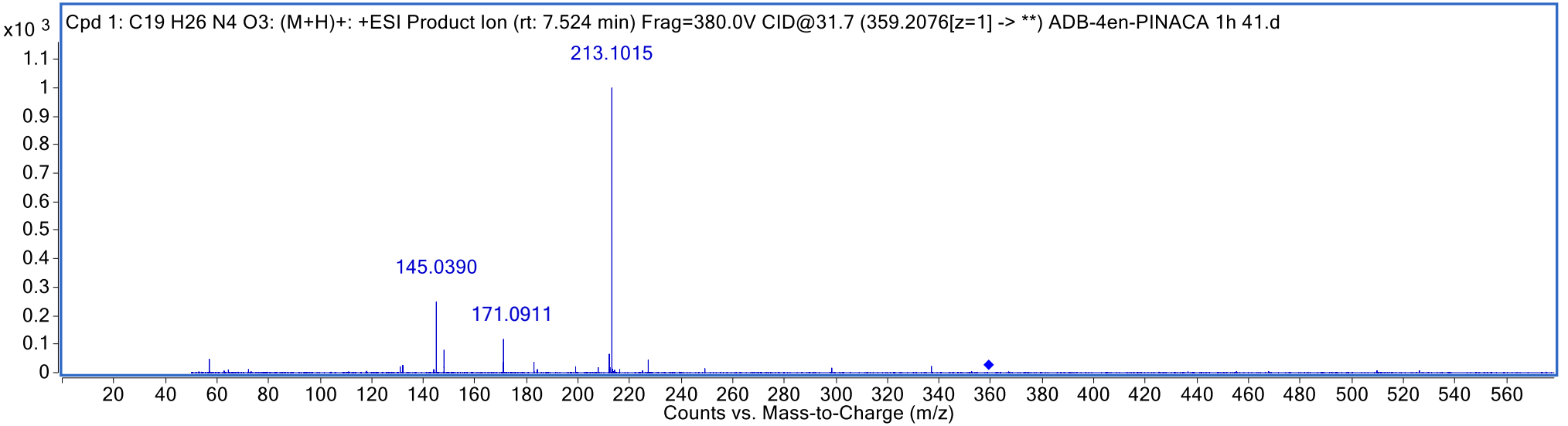
229.0972

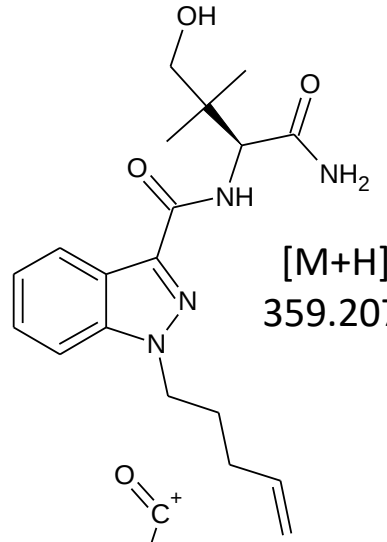


161.0346

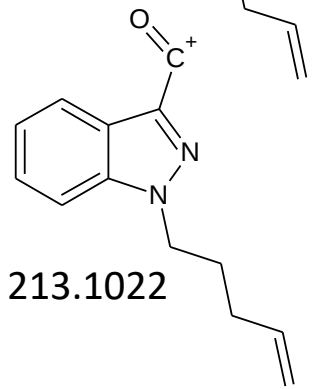
J5, Mono-hydroxylation (*tert*-butyl), RT 7.52 min, *m/z* 359.2077



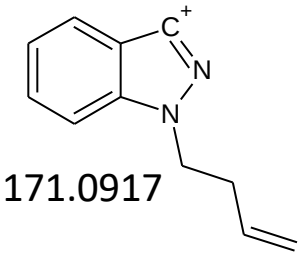




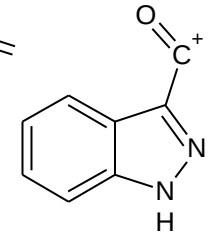
[M+H]+
359.2078



213.1022

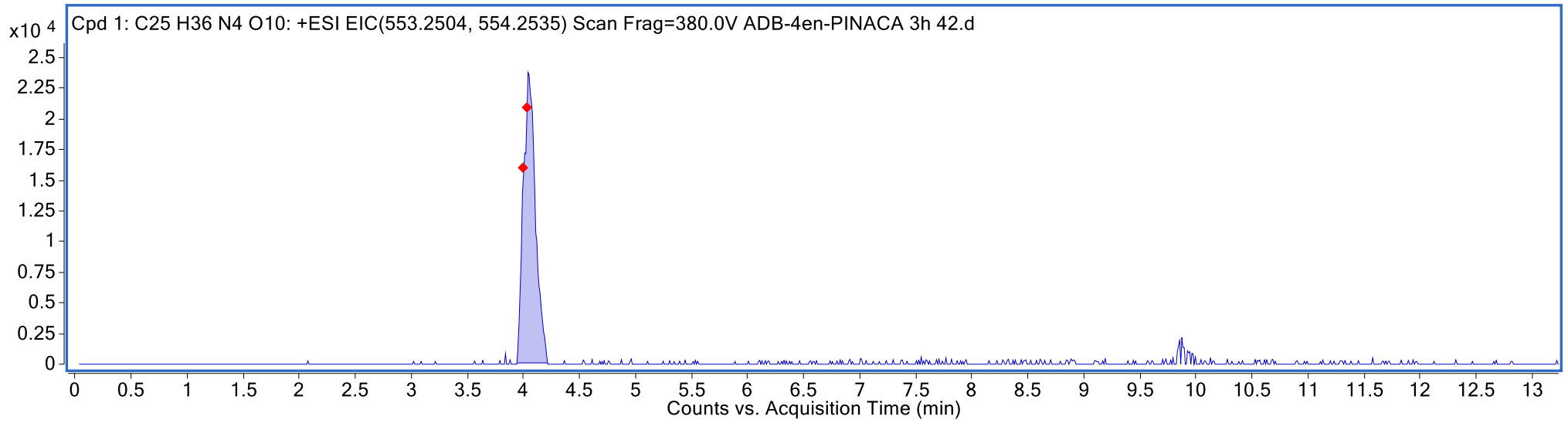


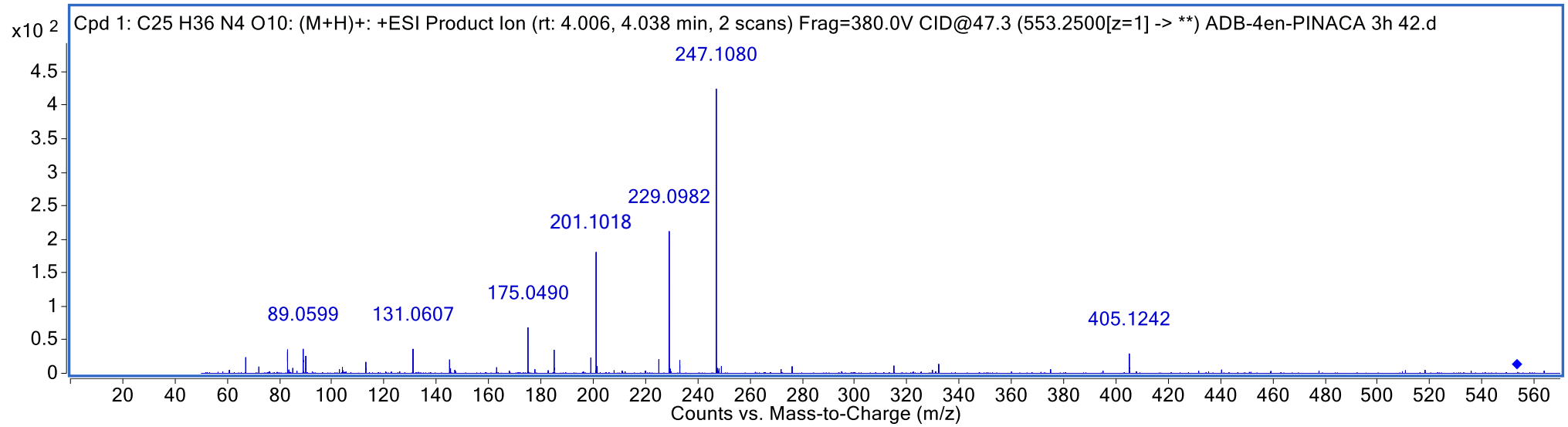
171.0917

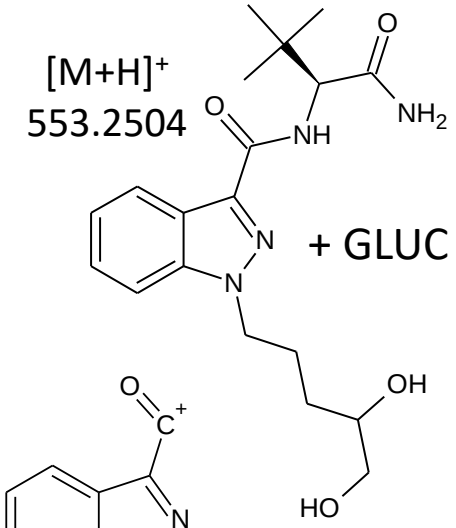


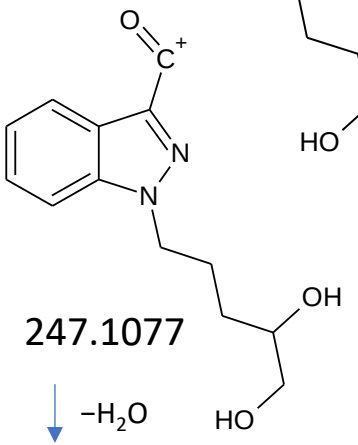
145.0396

J6, Dihydrodiol formation + glucuronidation, RT 4.05 min, *m/z* 553.2500

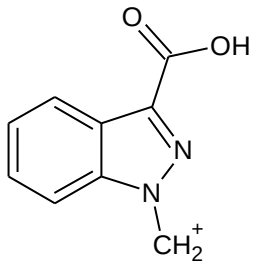


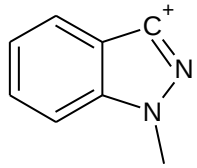




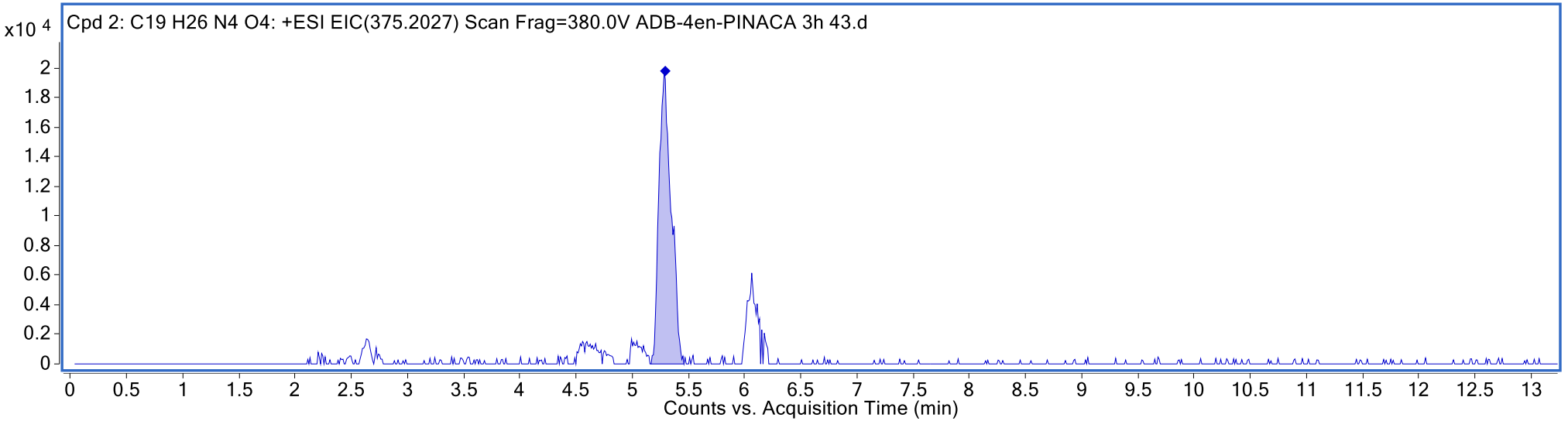


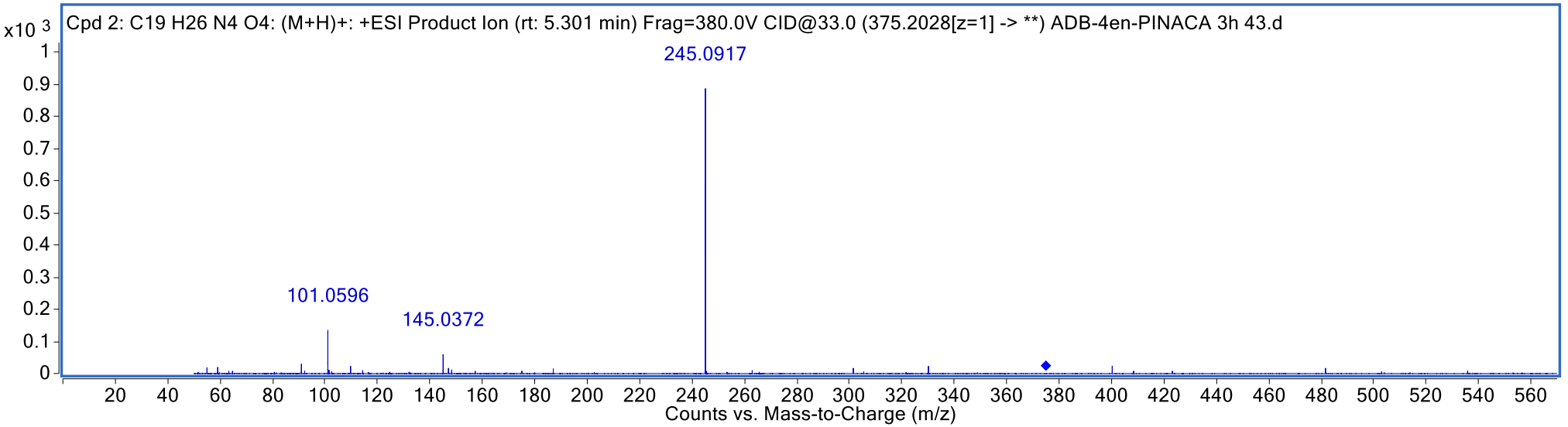
229.0972 → 201.1022 (−CO)

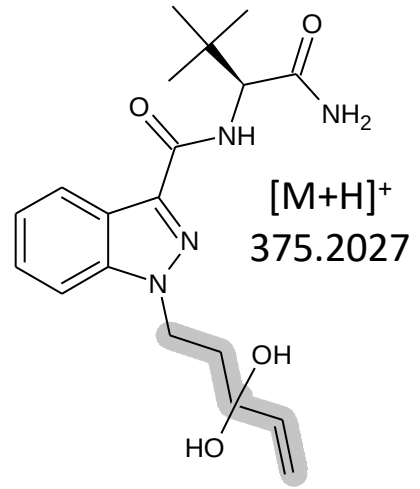


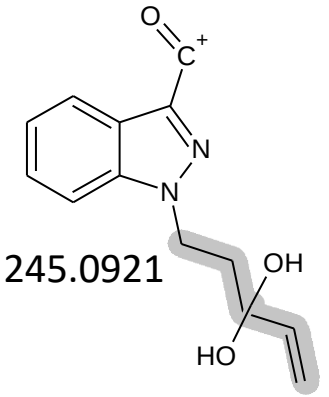


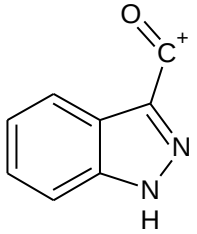
J7, Di-hydroxylation (pentenyl tail), RT 5.27 min, *m/z* 375.2021

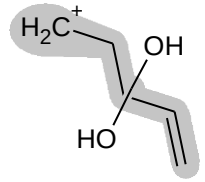




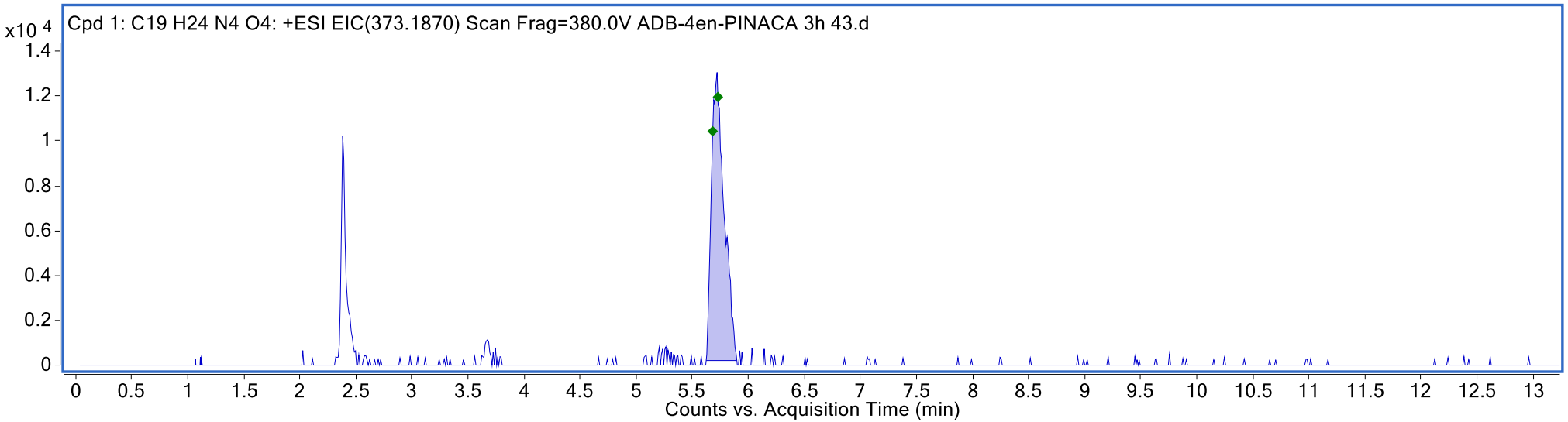


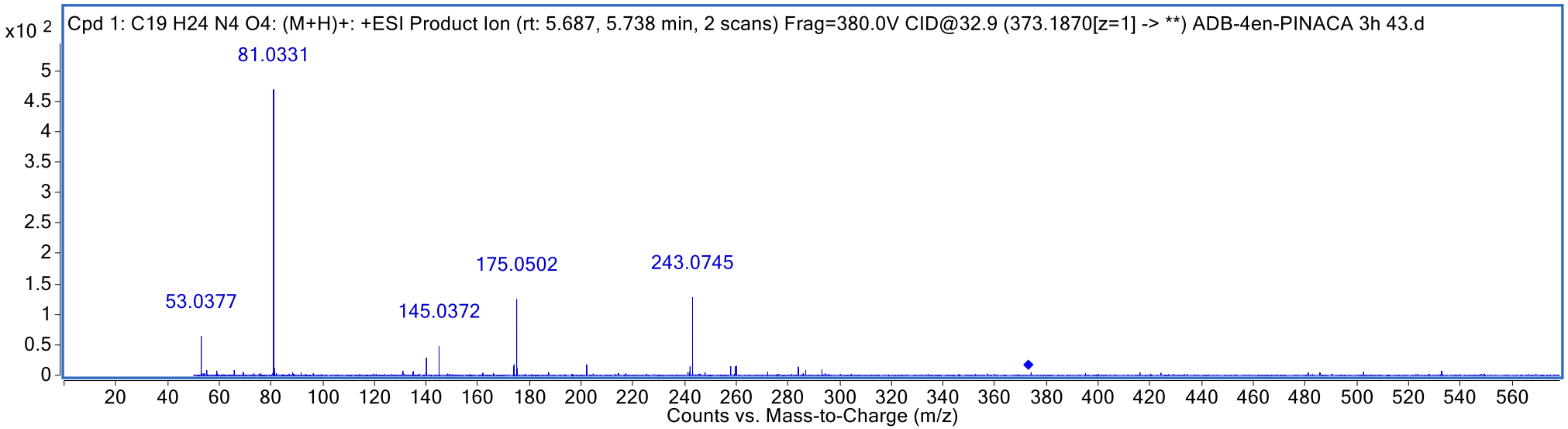


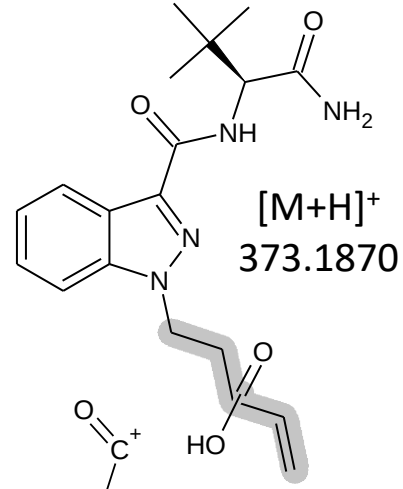


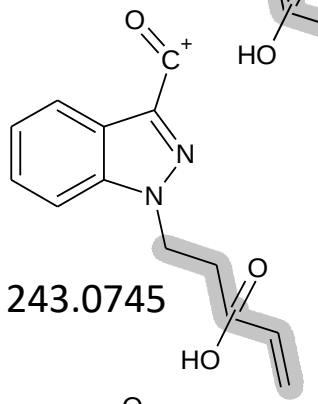


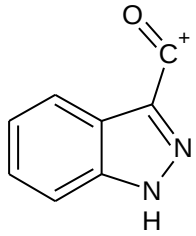
J8, Ketone formation + mono-hydroxylation (pentenyl tail), RT 5.71 min, *m/z* 373.1863

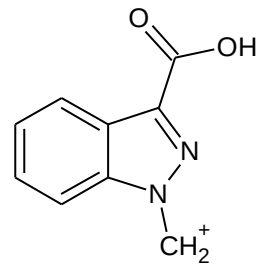


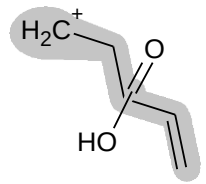




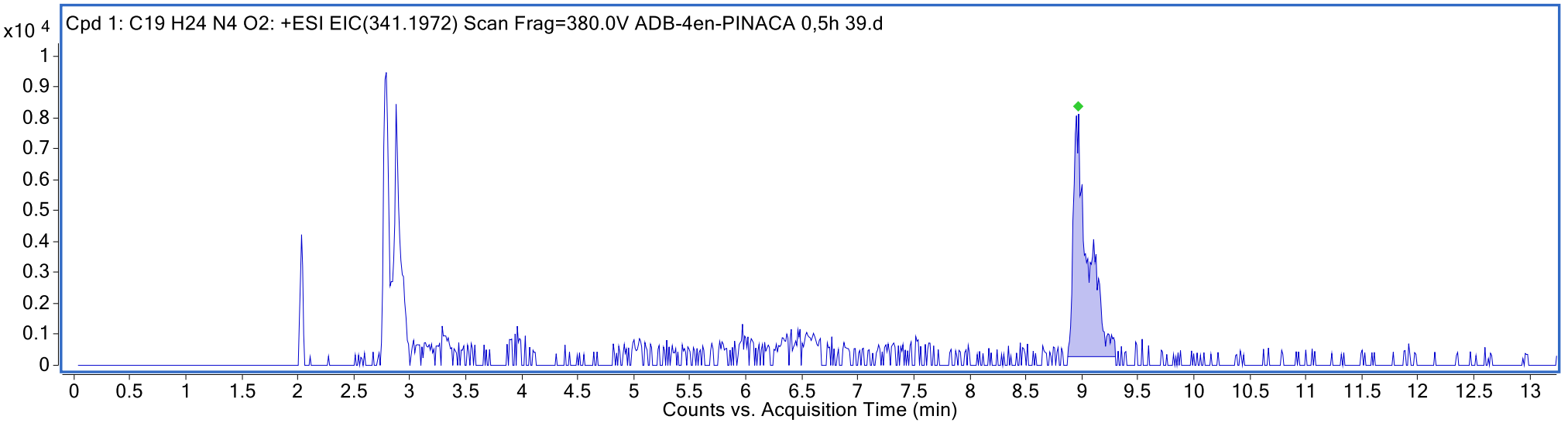


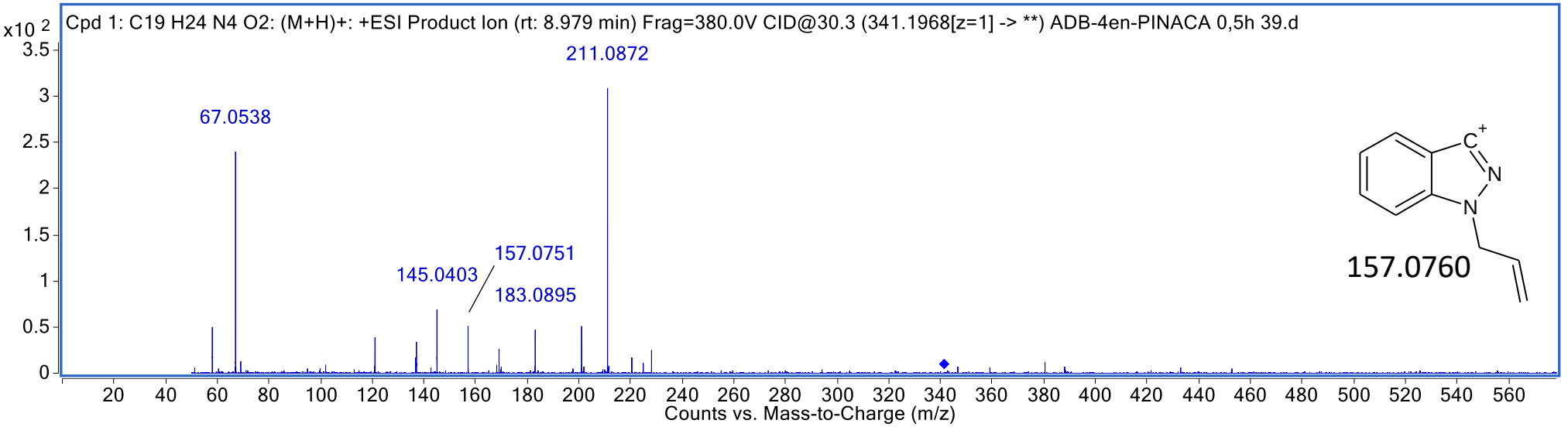


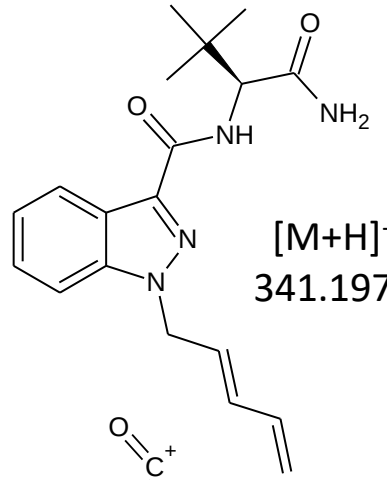


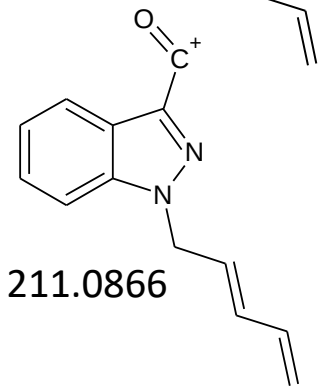


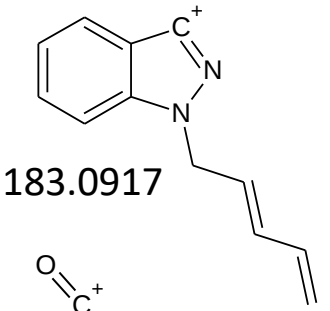
J9, Dehydrogenation (pentenyl tail), RT 8.97 min, *m/z* 341.1973

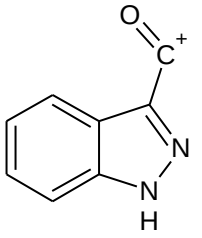


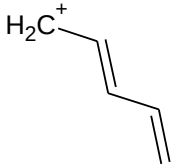




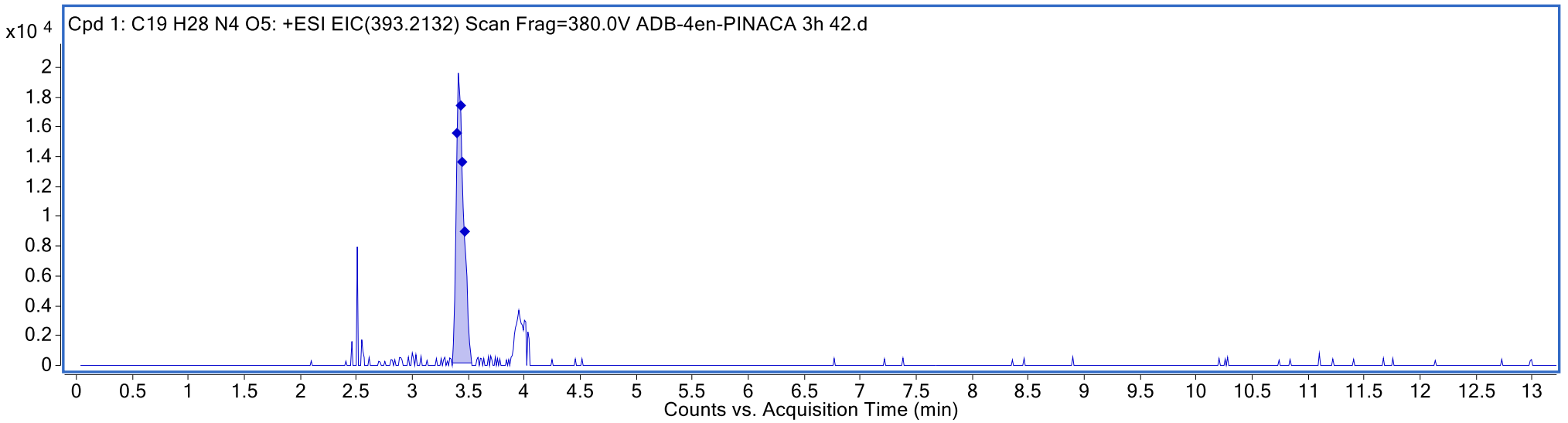


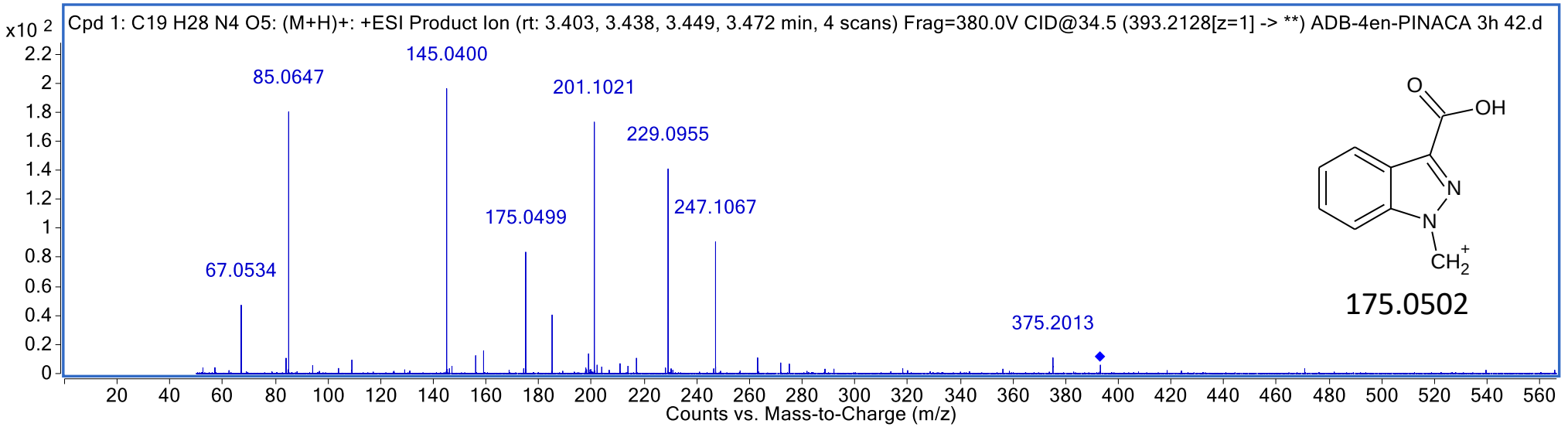


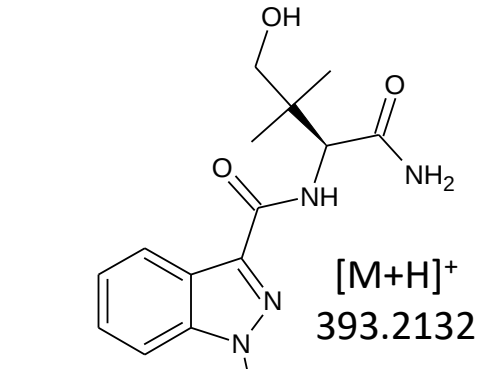




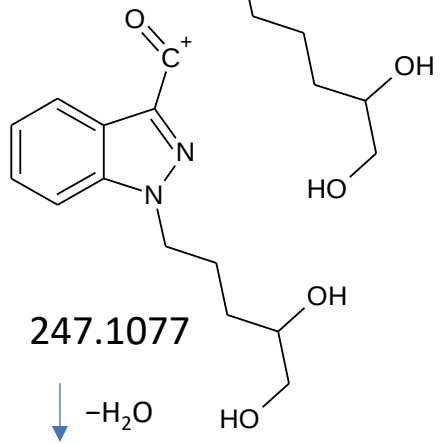
J10, Dihydrodiol formation + mono-hydroxylation (*tert*-butyl), RT 3.42 min, *m/z* 393.2126







[M+H]+
393.2132



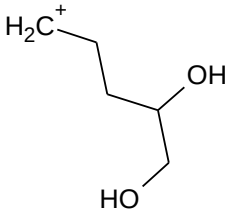
247.1077

$-H_2O$

229.0972 → 201.1022

$-CO$

175.0502

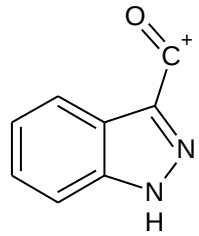


103.0754
(not detected)

$-H_2O$

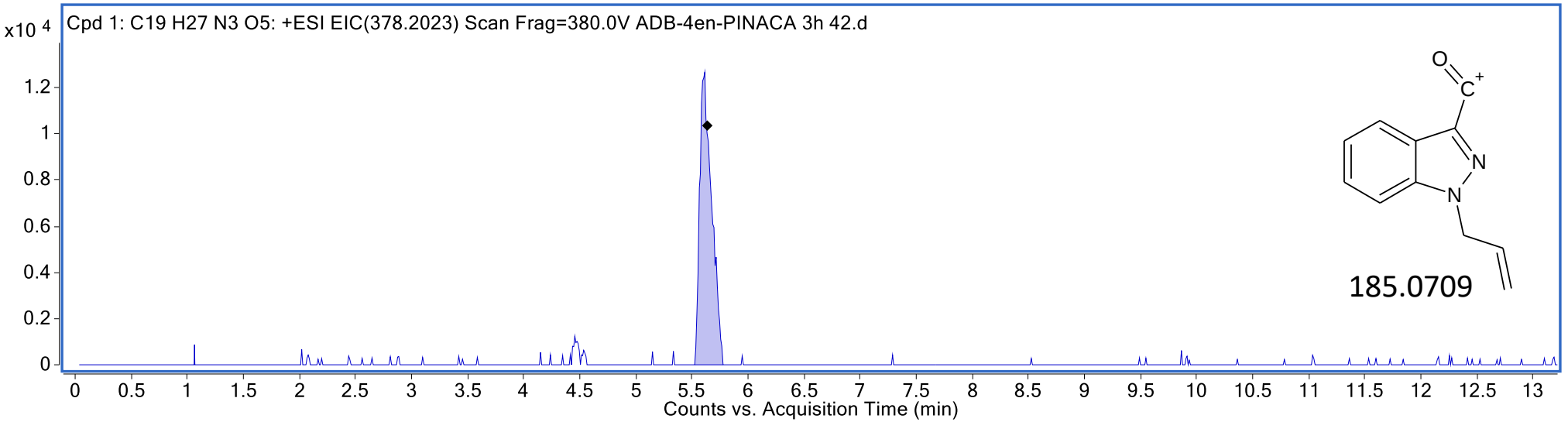
85.0648 → 67.0542

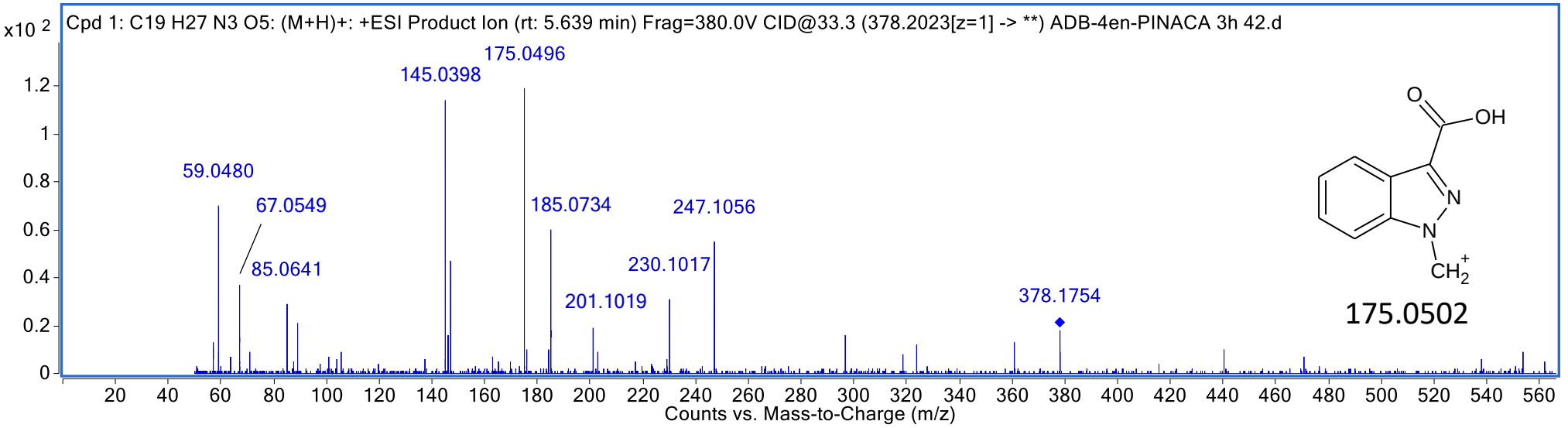
$-H_2O$

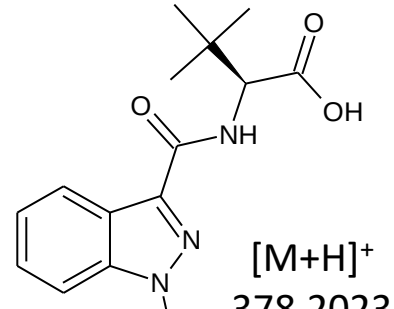


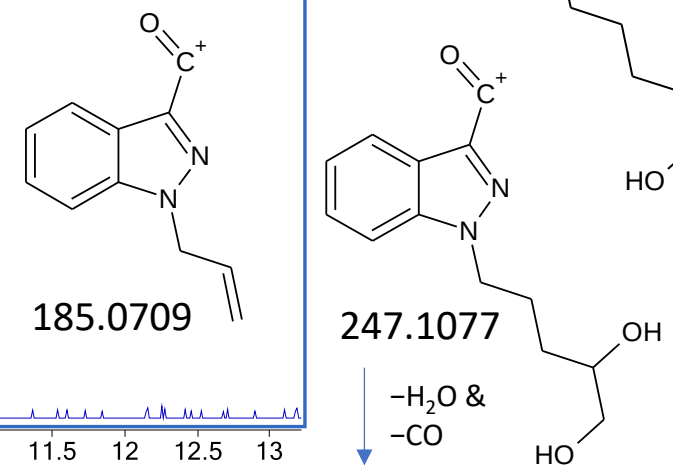
145.0396

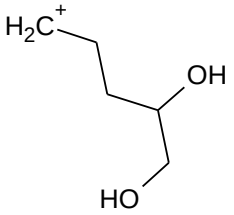
J11, Terminal amide hydrolysis + dihydrodiol formation, RT 5.60 min, *m/z* 378.2014

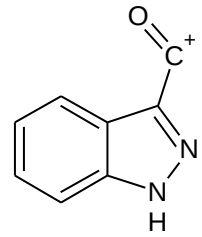


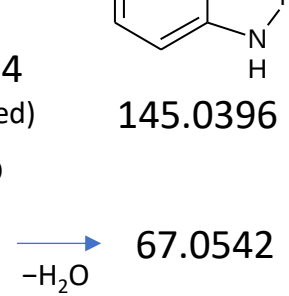








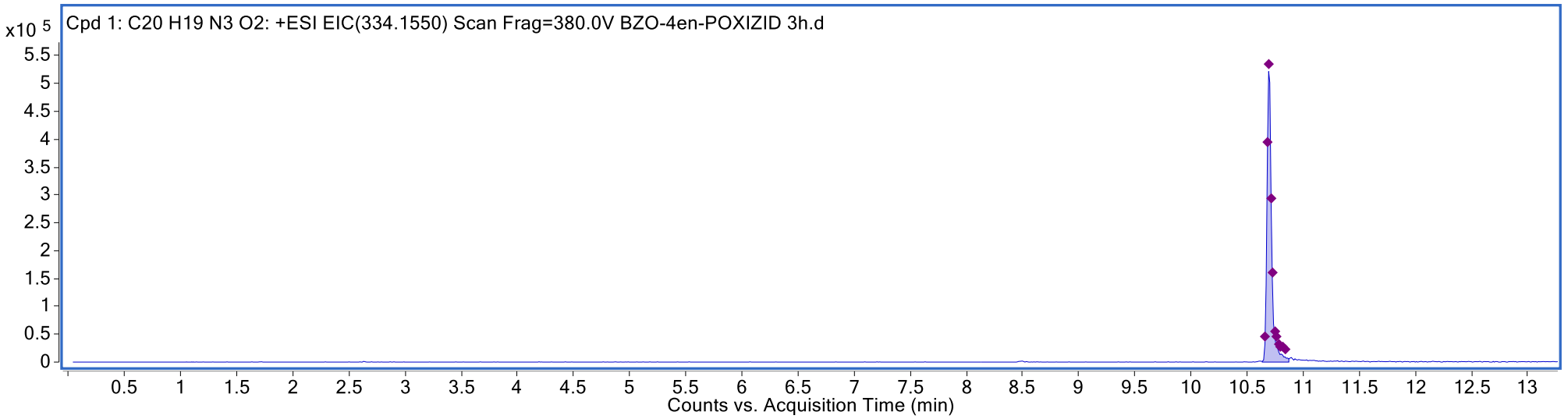


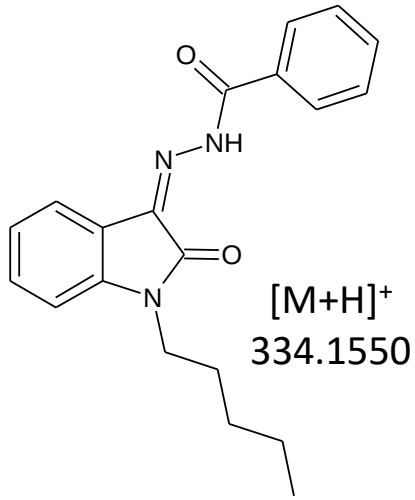


BZO-4en-POXIZID

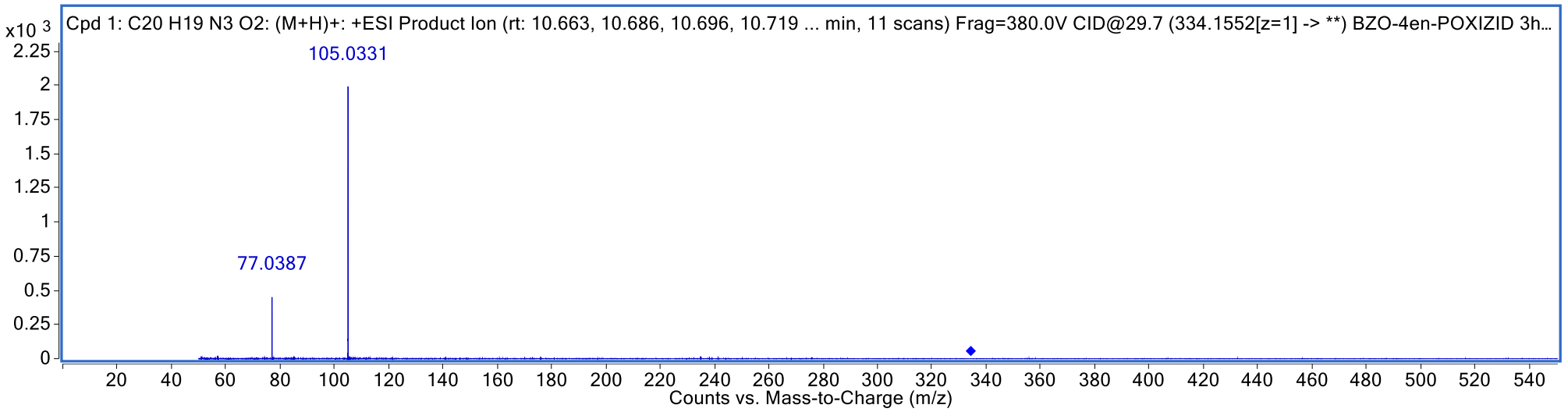
Metabolism

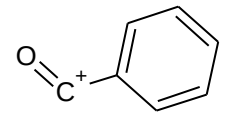
BZO-4en-POXIZID, RT 10.69 min, *m/z* 334.1563



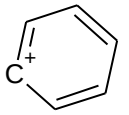


$[M+H]^+$
334.1550



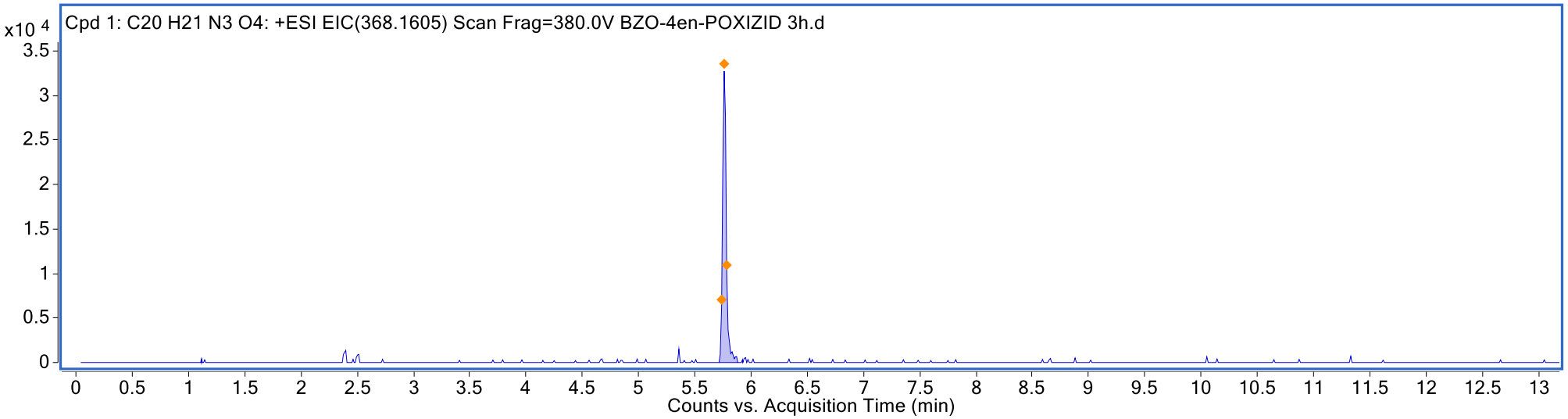


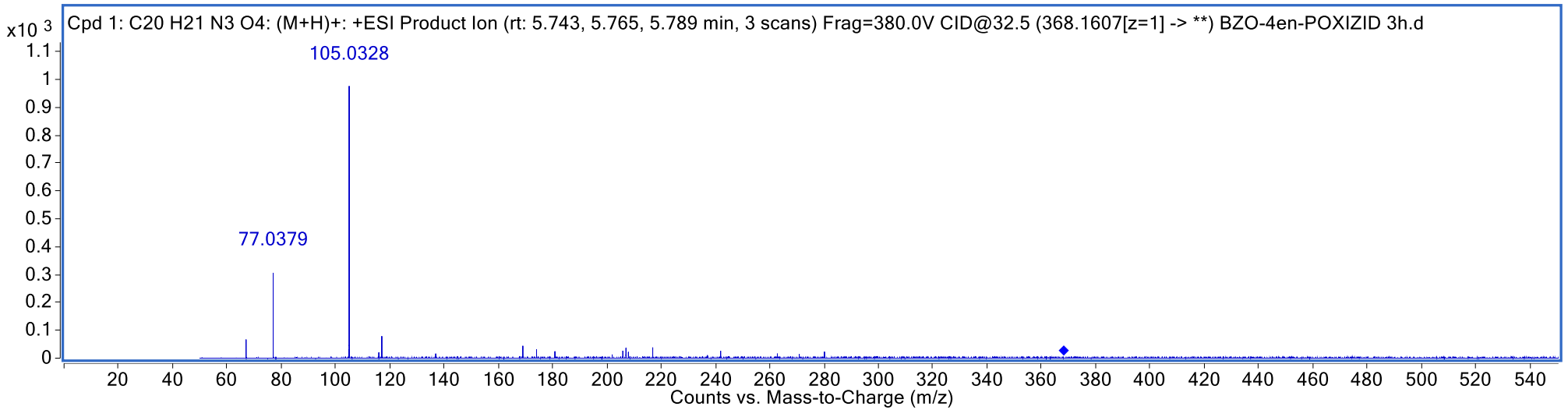
105.0335

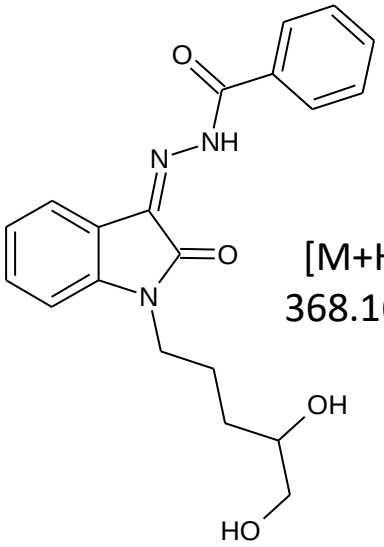


77.0386

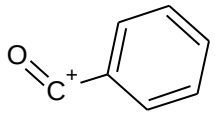
K1, Dihydrodiol formation, RT 5.76 min, *m/z* 368.1606



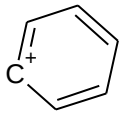




$[M+H]^+$
368.1605

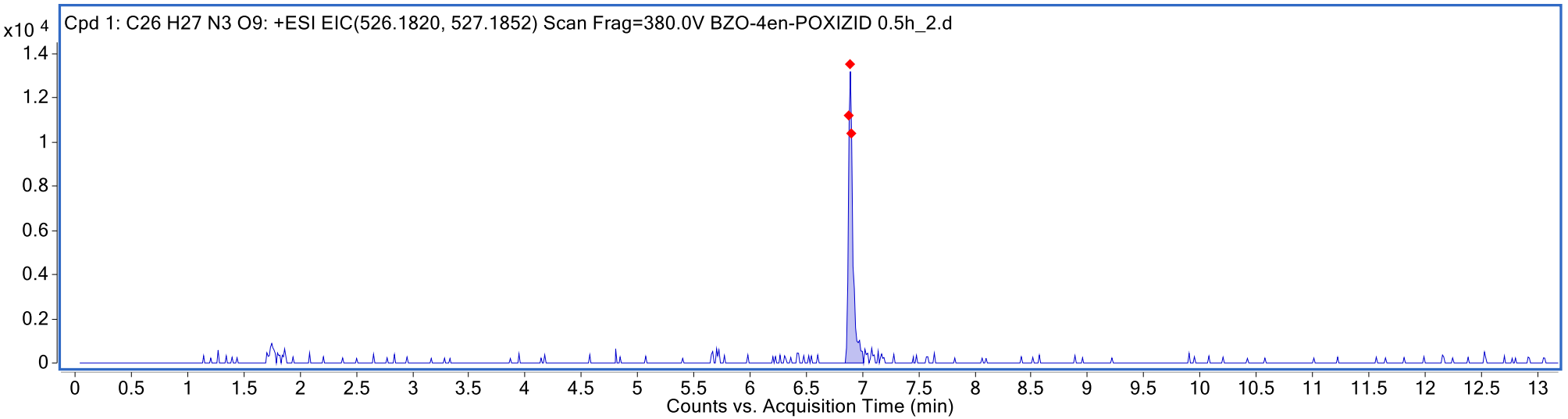


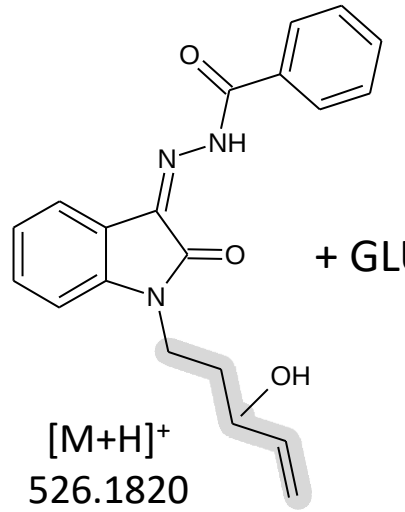
105.0335



77.0386

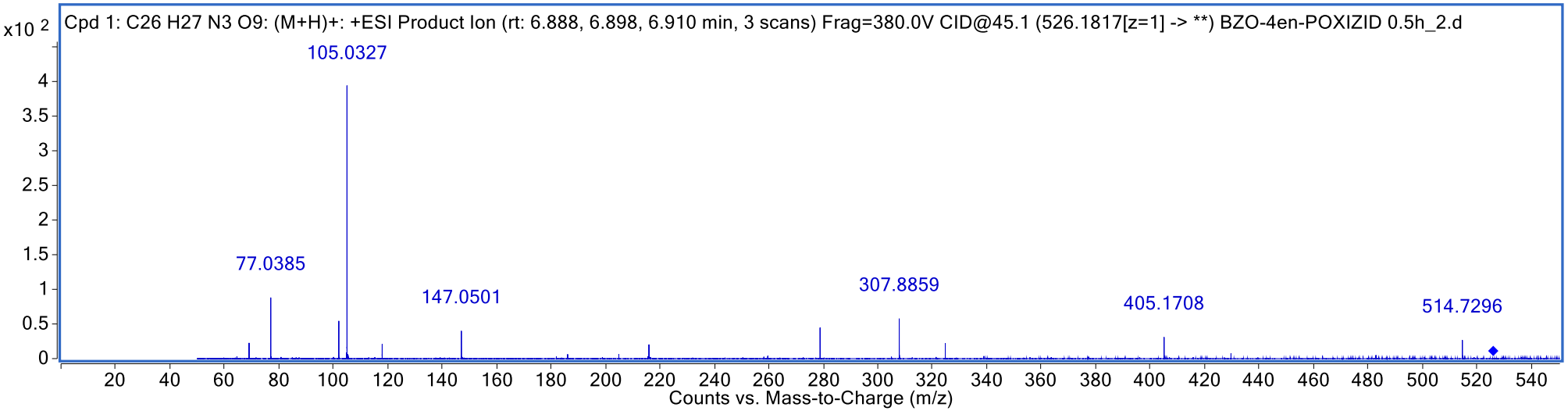
K2, Mono-hydroxylation (pentenyl tail) + glucuronidation, RT 6.89 min, *m/z* 526.1818

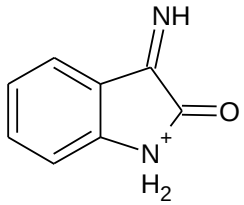




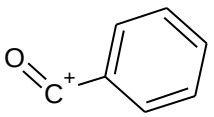
+ GLUC

$[M+H]^+$
526.1820

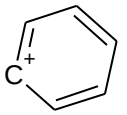




147.0553

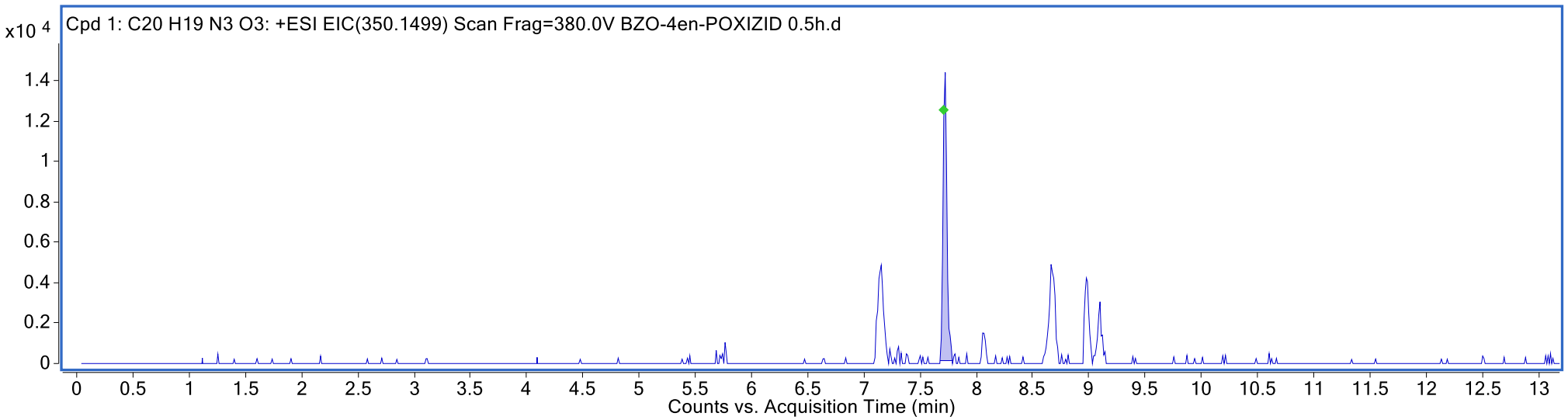


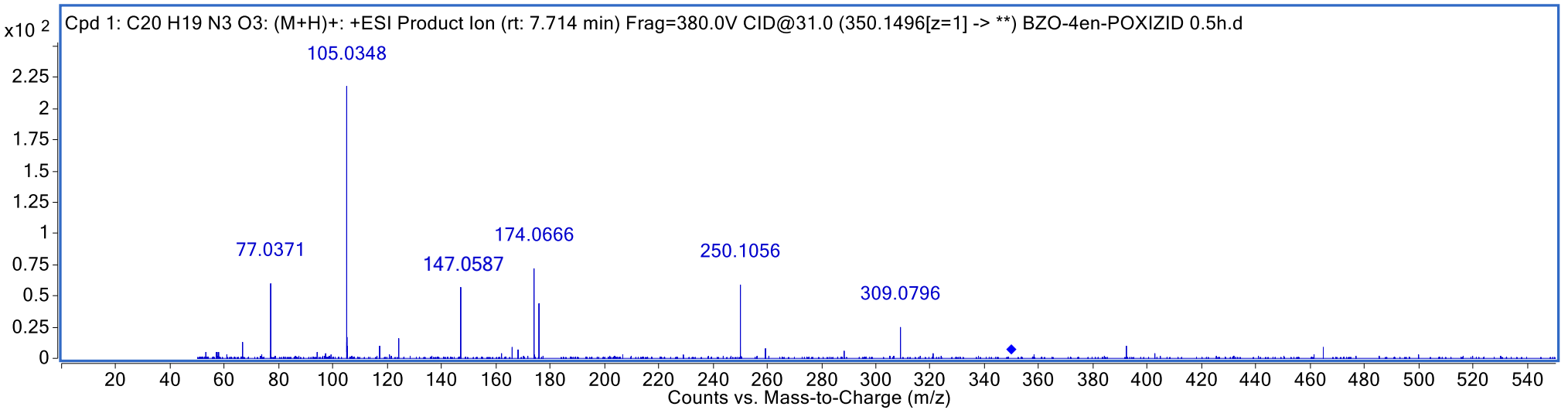
105.0335

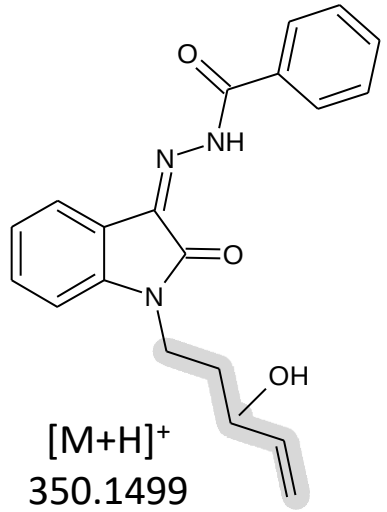


77.0386

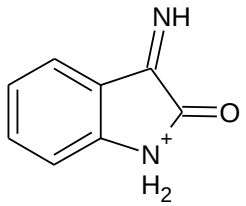
K3, Mono-hydroxylation (pentenyl tail), RT 7.73 min, *m/z* 350.1498



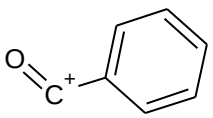




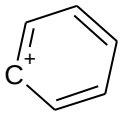
$[M+H]^+$
350.1499



147.0553



105.0335



77.0386