

UHPLC–QTOF–MS/MS Based Metabolic Profiling of Frankincense Aqueous Extract and its Synergistic Anticancer Effect with Sorafenib in HepG2 Cells.

Wesam Ragab^{1*+}, Kamel Mahmoud¹⁺, Seham Salah El-Din², Osama M. Gomaa², Rasha M. Allam³, Abeer Sayed⁴, Rabab Mohammed^{4*}.

¹Pharmacognosy Department, Faculty of Pharmacy, MUST University, 6th October City, Giza 12566, Egypt.

²Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.

³Pharmacology Department, Medical and Clinical Research Institute, National Research Centre, 33 El-Bohouth St., Dokki, P.O.12622, Cairo, Egypt.

⁴Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt.

* Corresponding authors: wesam.ragab@must.edu.eg

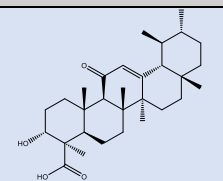
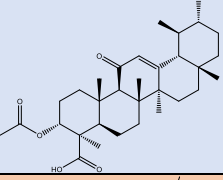
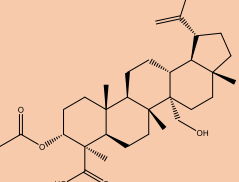
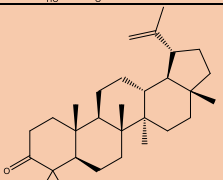
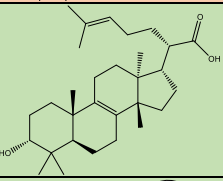
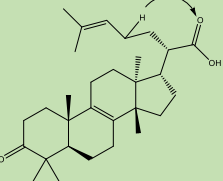
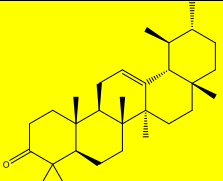
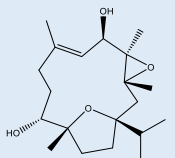
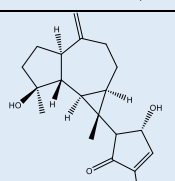
Orcid no. <https://orcid.org/0000-0002-1809-8005>

rababmohammed@pharm.bsu.edu.eg

<https://orcid.org/0000-0001-9683-4250>

⁺ These authors contributed equally to this work.

Suppl. Table (1): PubChem IDs, structures, and docking scores of docked ligands.

					Apoptosis		Autophagy	
	Class	Name	PubChem ID	Structure	BCL-2	P53	mTOR	LC3C
		Internal ligand			-12.3	-7.3	-5.6	-3.8
1	Ursane triterpene	11-Keto boswellic acid	9847548		-8.4	-5.7	-5.2	-5.2
2		3-O-Acetyl 11-keto boswellic acid	11168203		-8.2	-5.4	-4.7	0.8
3	Lupane triterpene	3-Acetyl-27-hydroxy-lupeolic acid	21575462		-7.3	-5.1	-4.3	0.5
4		Lupenone	92158		-8.1	-5.5	-6.0	-4.2
5	Tricullane triterpene	3-hydroxytirucallic acid (α -Elemolic acid)	441677		-7.7	-6.2	-5.3	-4.5
6		3-oxo-tirucallic acid (β -Elemonic acid)	15559100		-7.8	-6.2	-5.2	-4.2
7	Oleane triterpene	α -Amyrenone	12306155		-8.9	-5.6	-5.6	-4.7
8	Diterpene	Boscartin C	319538644		-7.6	-4.5	-3.9	-5.0
9		Boscartol G	275376549		-7.5	-5.4	-5.2	-6.0

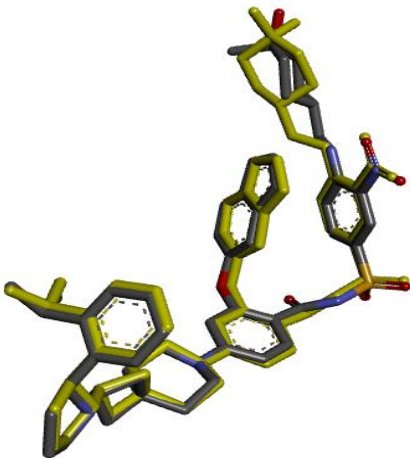
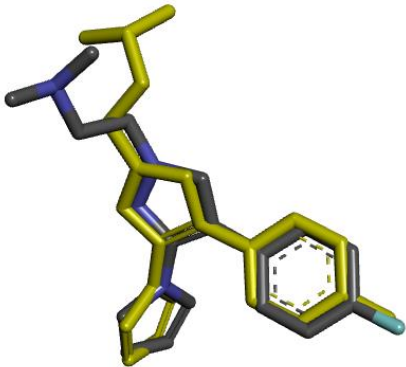
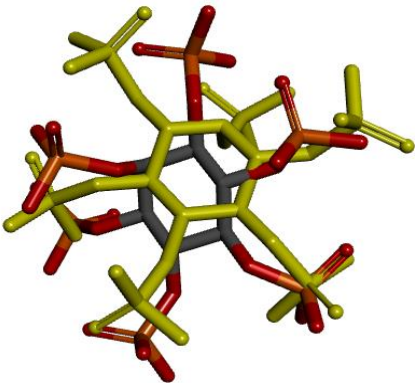
Suppl. Table (2): Docking scores, different amino acids bonded to docked compounds along with types of interaction in the active sites of BCL-2 and P53.

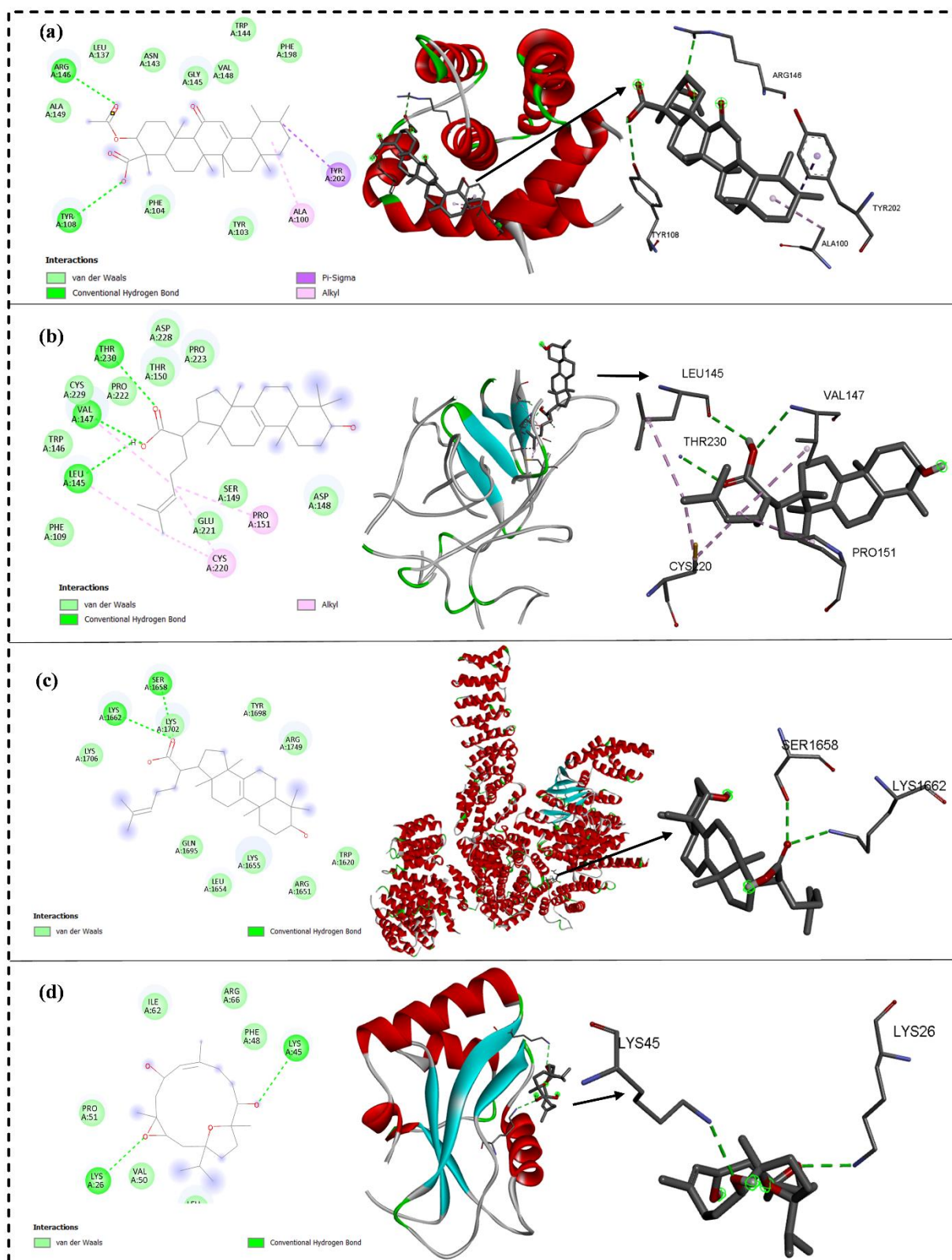
Apoptosis markers							
		BCL-2 (8HOI)			P53 (3ZME)		
	Compound	Docking Score (kcal/mol)	Amino acid	Type of interaction	Docking Score (kcal/mol)	Amino acid	Type of interaction
	Internal ligand	-12.3	ARG146 ALA100 <u>TYR202</u> <u>TYR103</u> GLN A99 PHE112 MET115	π -alkyl C-H bond π - π stacked π -alkyl Conventional H-bond π -sigma π -sigma	-7.3	ASP228 PRO223* <u>VAL147</u> <u>LEU145</u> CYS220 PRO151	Halogen (Fluorine) π -alkyl π -alkyl π -alkyl π -Donor H-bond π -Donor H-bond
1	Keto boswellic acid	-8.4	<u>TYR202</u>	Conventional H-bond	-5.7	PRO223* PRO222	Carbon H-bond Alkyl
2	Acetyl keto boswellic acid	-8.2	ARG146 TYR108 <u>TYR202</u> ALA100	Conventional H-bond Conventional H-bond π -sigma Alkyl	-5.4	PRO223*	Carbon H-bond
3	3-Acetyl-27-hydroxy-lupeolic acid	-7.3	TYR108	Conventional H-bond π -sigma	-5.1	PRO222 THR150	Alkyl Conventional H-bond
4	Lupenone	-8.1		multiple Van der Waals forces	-5.5	PRO222	Alkyl
5	3-hydroxytirucallic acid (α -Elemolic acid)	-7.7	PHE104	π -alkyl	-6.2	THR230 <u>VAL147</u> <u>LEU145</u> PRO151 CYS220	Conventional H-bond Conventional H-bond Conventional H-bond Alkyl Alkyl
6	3-oxo-tirucallic acid (β -Elemonic acid)	-7.8	PHE104	π -alkyl	-6.2	THR230 <u>VAL147</u> PRO151 CYS220 LEU145	Conventional H-bond Alkyl Alkyl Alkyl Unfavorable acceptor-acceptor
7	α -Amyrenone	-8.9		multiple Van der Waals forces	-5.6	THR150	Conventional H-bond
8	Boscartin C	-7.6	PHE112 MET115	π -sigma Alkyl	-4.5	THR150 ASP148	Conventional H-bond Unfavorable acceptor-acceptor
9	Boscartol G	-7.5	ALA100 <u>TYR103</u> <u>TYR202</u>	Alkyl & π -alkyl Alkyl & π -alkyl π donor H-bond	-5.4	THR150 <u>VAL147</u> PRO223*	Conventional H-bond Conventional H-bond Alkyl

Suppl. Table (3): Docking scores, different amino acids bonded to docked compounds along with types of interaction in the active sites of mTOR and LC3C.

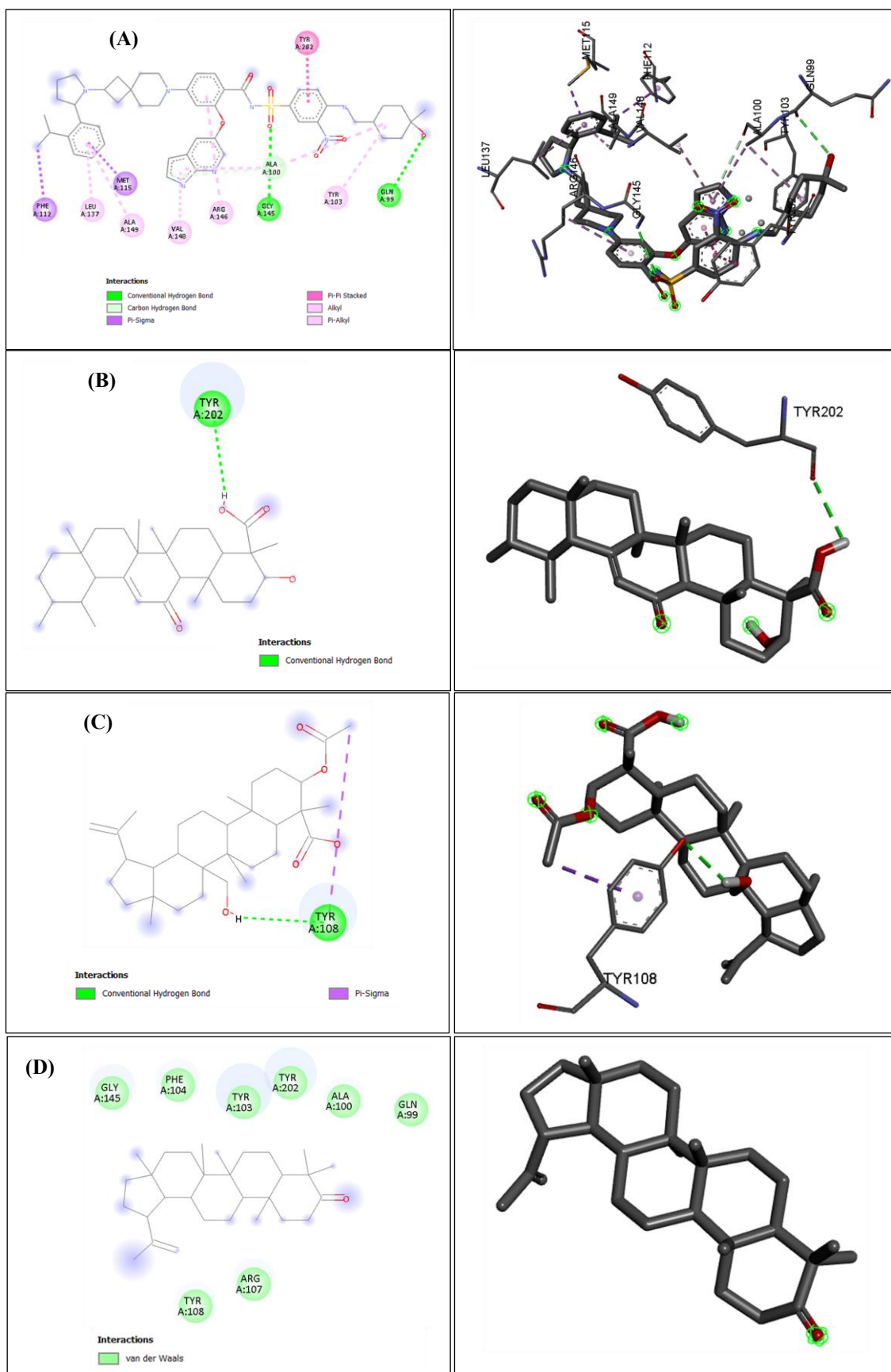
		Autophagy marker					
		mTOR (9F44)			LC3C (3WAM)		
	Compound	Docking Score (kcal/mol)	Amino acid	Type of interaction	Docking Score (kcal/mol)	Amino acid	Type of interaction
	Internal ligand	-5.6	LYS1662 LYS1655 <u>SRE1658</u> TYR1698 <u>LYS1702</u> LYS1788 ARG1749	Conventional H-bond Salt bridge Salt bridge Conventional H-bond Conventional H-bond Salt bridge Conventional H-bond Salt bridge Salt bridge	-3.8	LEU A49 LYS A45 ARG A66	Conventional H-bond Conventional H-bond Conventional H-bond Salt bridge Conventional H-bond Salt bridge
1	Keto boswellic acid	-5.2	LYS1655	Conventional H-bond	-5.2	LYS A45 PRO A51	Unfavorable donor-donor Alkyl
2	Acetyl keto boswellic acid	-4.7	ARG1749 LYS1662	Conventional H-bond Alkyl	0.8		
3	3-Acetyl-27-hydroxy-lupeolic acid	-4.3	LYS1788 ARG1651	Conventional H-bond Alkyl	0.5		
4	Lupenone	-6.0	LYS1662	Alkyl	-4.2	LEU A49	Alkyl
5	3-hydroxytirucallic acid (α -Elemolic acid)	-5.3	LYS1662 <u>SRE1658</u>	Conventional H-bond Conventional H-bond	-4.5	LYS A45	Conventional H-bond
6	3-oxo-tirucallic acid (β -Elemonic acid)	-5.2	GLN1695 ARG1651 TRP1620 LYS1655 ARG1749	Conventional H-bond Alkyl π -alkyl Alkyl Unfavorable donor-donor	-4.2	SER A65	Conventional H-bond
7	α -Amyrenone	-5.6		multiple Van der Waals forces	-4.7	PRO A51	Alkyl
8	Boscartin C	-3.9		multiple Van der Waals forces	-5.0	LYS A45 LYS A26	Conventional H-bond Conventional H-bond
9	Boscartol G	-5.2	<u>LYS1702</u>	Alkyl	-6.0	LEU A49 ILE A62	Unfavorable donor-donor Alkyl

Suppl. Table (4): Alignment of co-crystallized ligand with the docked ligand pose (yellow).

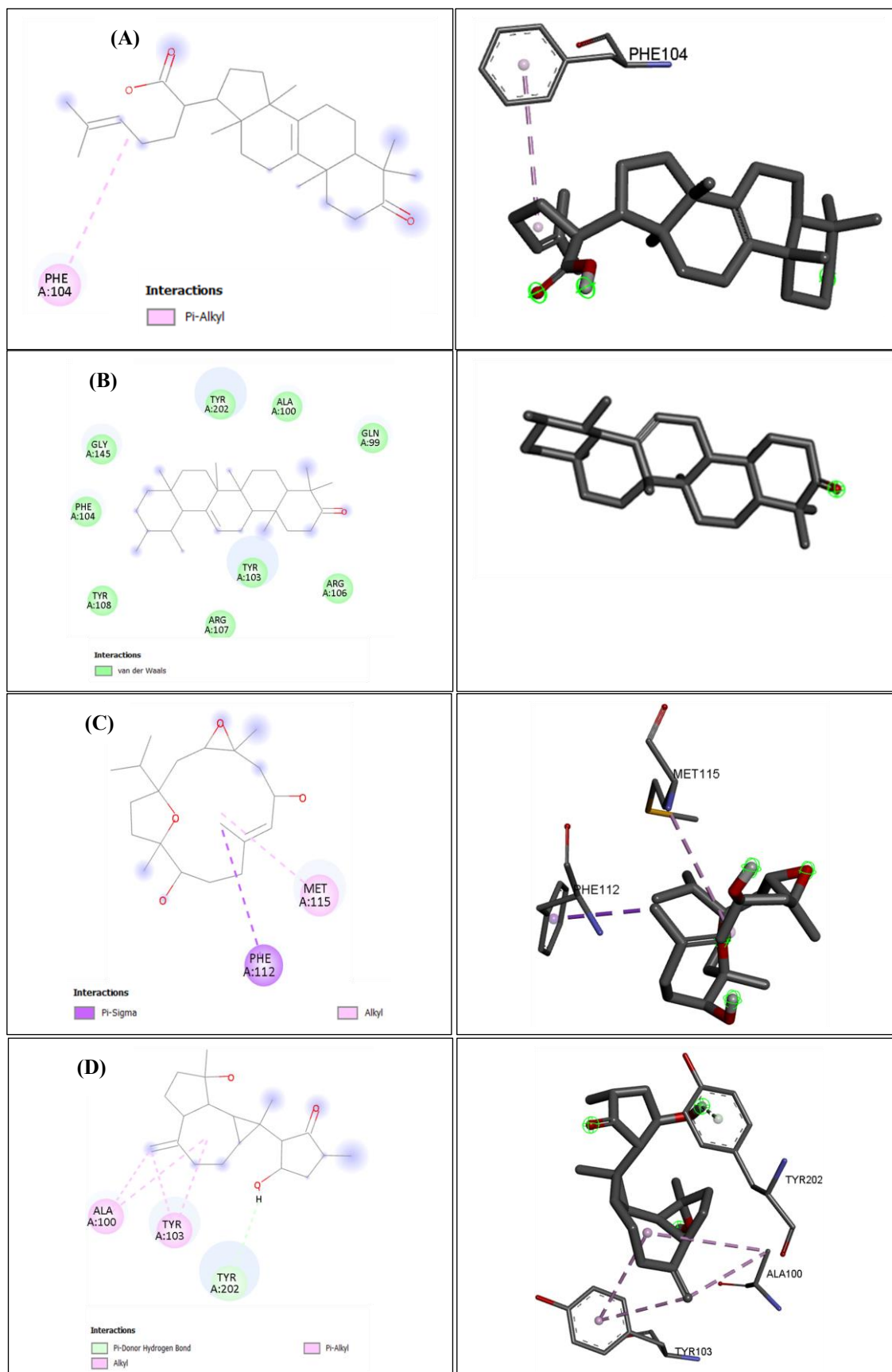
BCL-2	P53
	
RMSD: 0.724	RMSD: 1.361
mTOR	LC3C
	
RMSD: 2.690	RMSD: 1.901



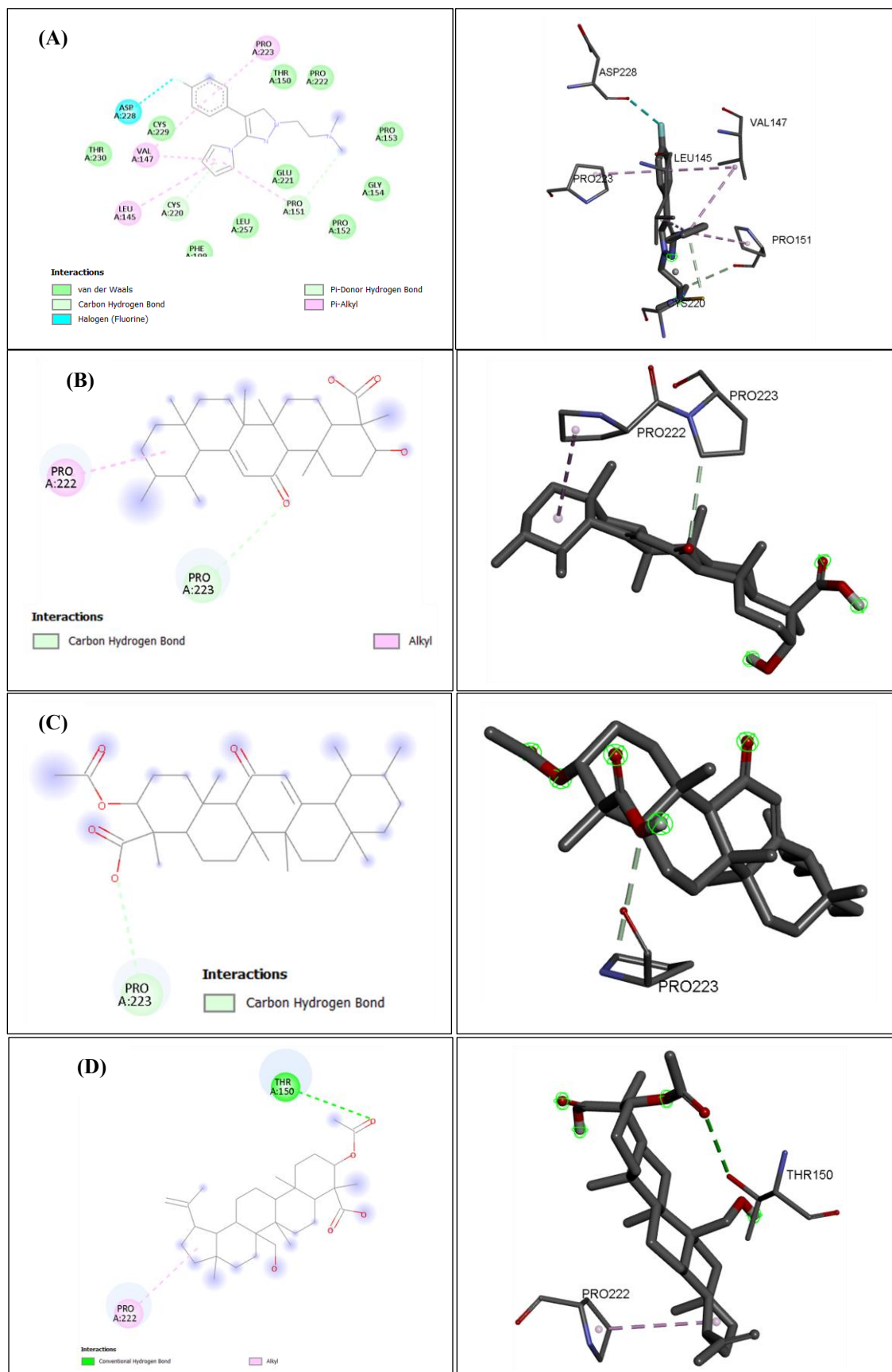
Suppl. Fig. 1. 2D and 3D pictures representing the Binding interactions of compounds with the highest binding energies: **(a)** 3-O-acetyl 11-keto boswellic acid in the active site of BCL-2, **(b)** α-Elemolic acid in the active site of P53, **(c)** α-Elemolic acid in the active site of mTOR, and **(d)** Boscartin C in the active site of LC3C.



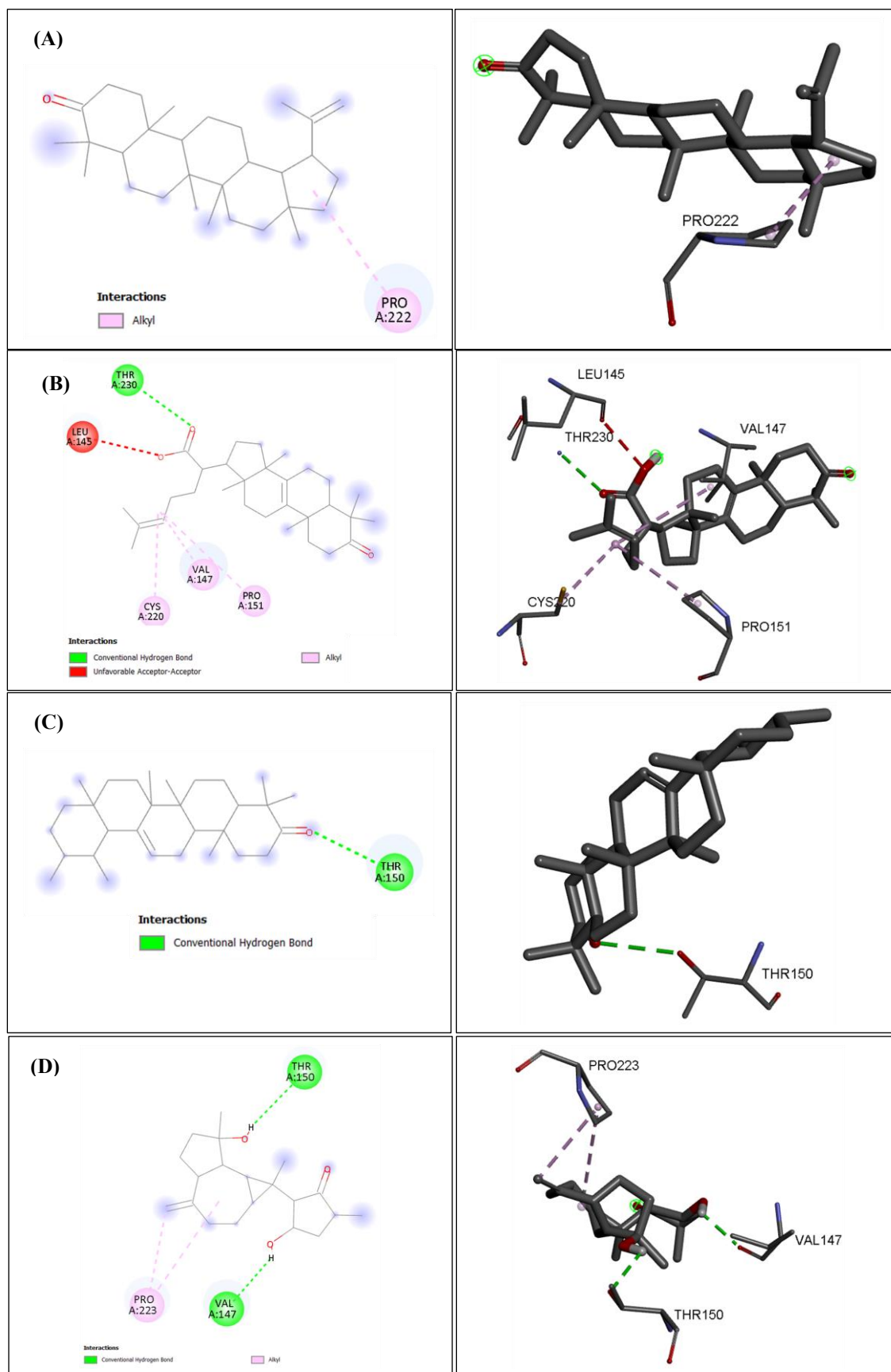
Suppl. Fig. 2. 2D and 3D pictures representing the Binding interactions of (A) Internal ligand, (B) 11-Keto boswellic acid, (C) 3-Acetyl-27-hydroxy-lupeolic acid, and (D) Lupenone in the active site of Bcl-2.



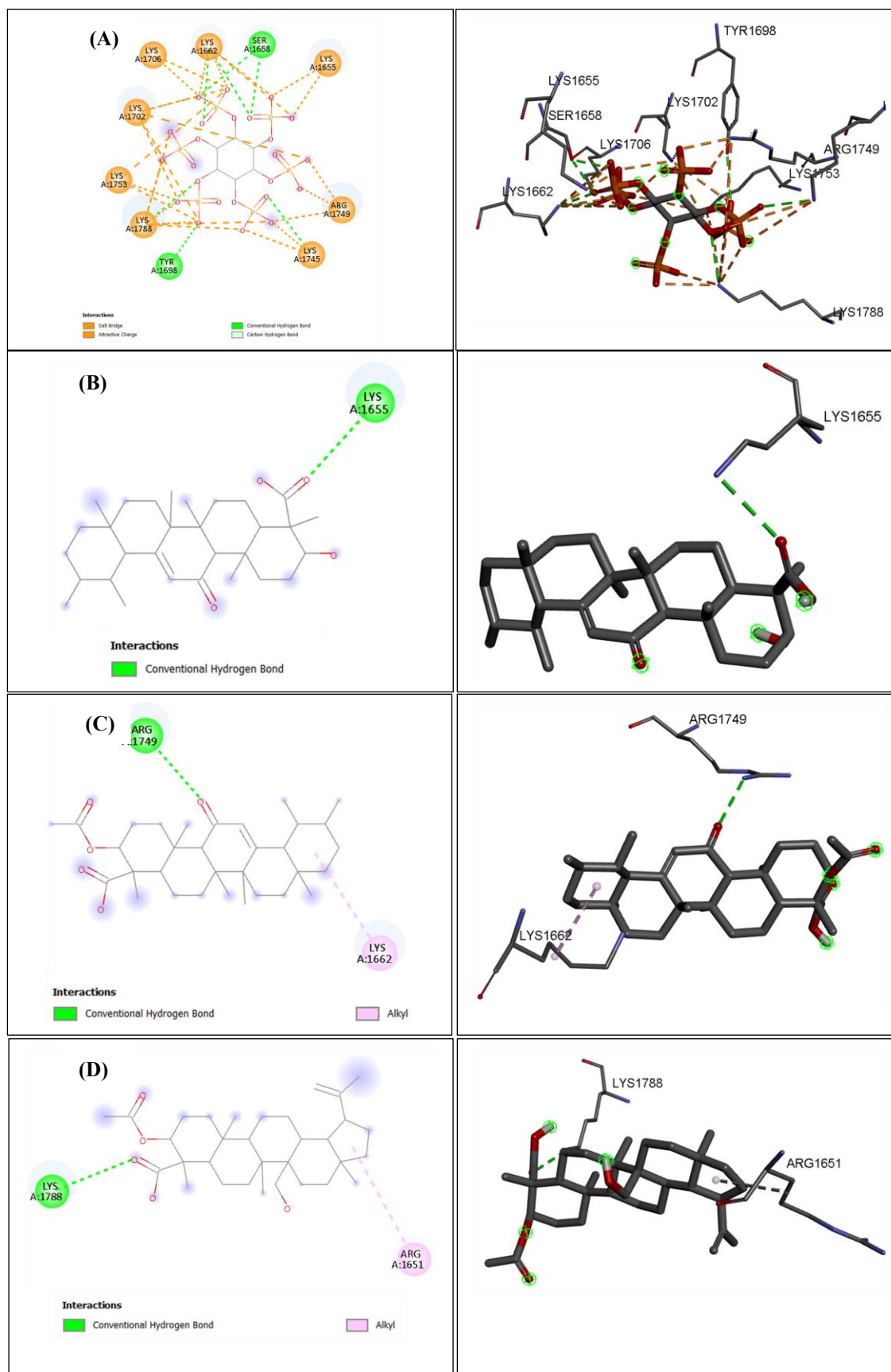
Suppl. Fig. 3. 2D and 3D pictures representing the Binding interactions of (A) 3-oxo-tirucallic acid, (B), α -Amyrenone (C) Boscartin C, and (D) Boscartol G in the active site of Bcl-2.



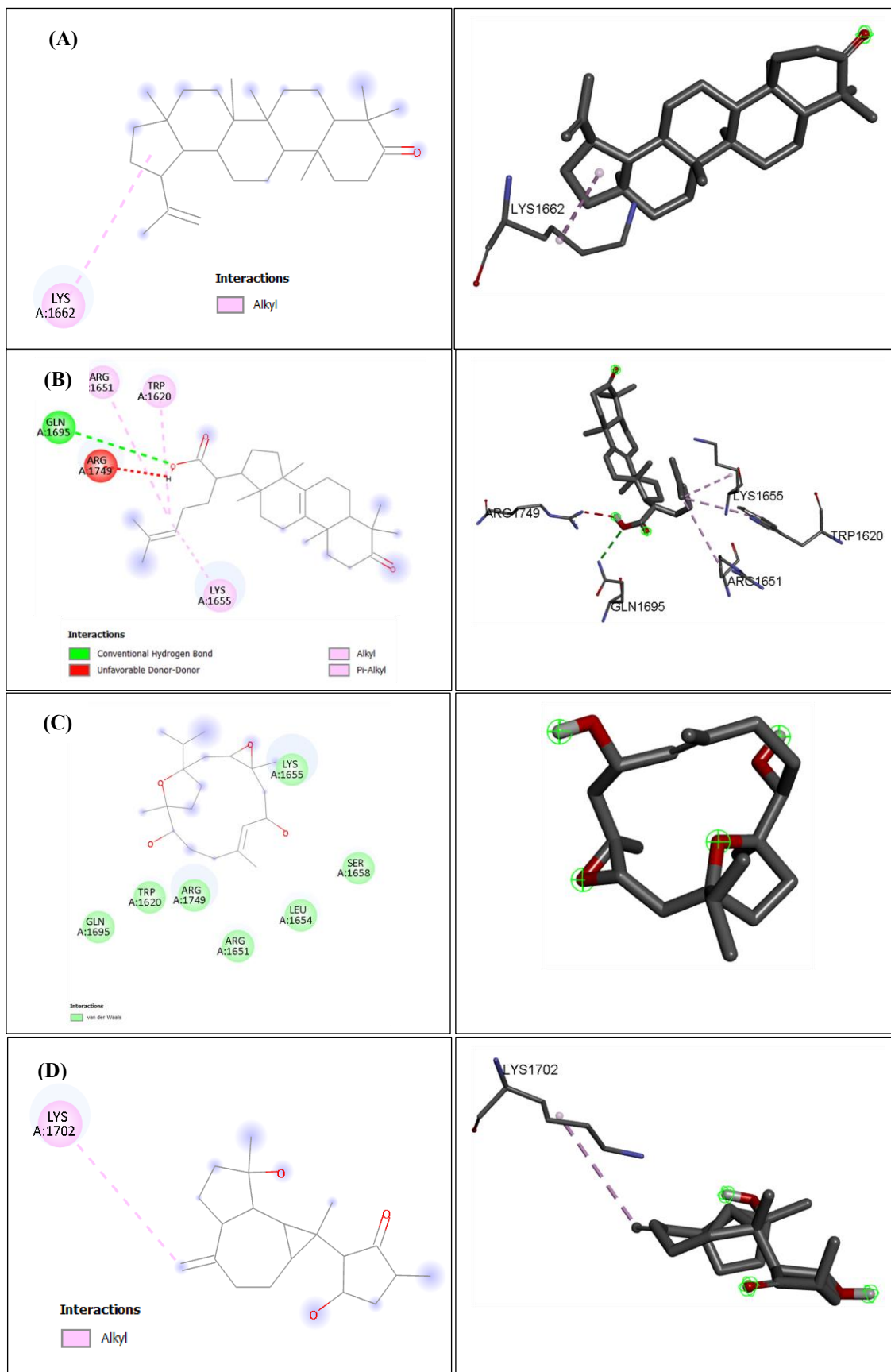
Suppl. Fig. 4. 2D and 3D pictures representing the Binding interactions of (A) Internal ligand, (B) 11-Keto boswellic acid, (C) 3-O-Acetyl 11-keto boswellic acid, and (D) 3-Acetyl-27-hydroxy-lupeolic acid in the active site of P53.



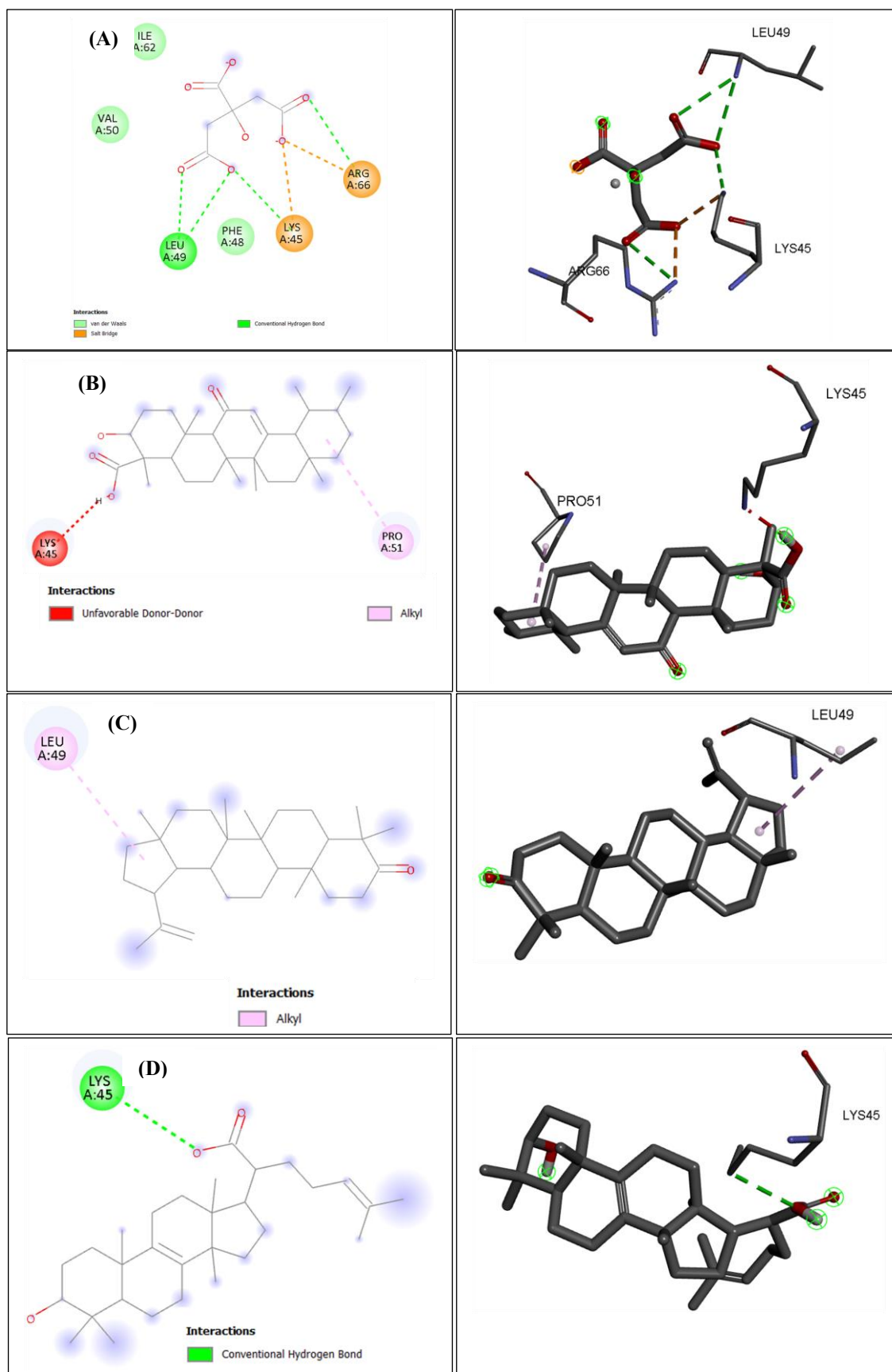
Suppl. Fig. 5. 2D and 3D pictures representing the Binding interactions of (A) Lupenone, (B) 3-oxo-tirucallic acid, (C) α -Amyrenone, and (D) Boscartol G in the active site of P53.



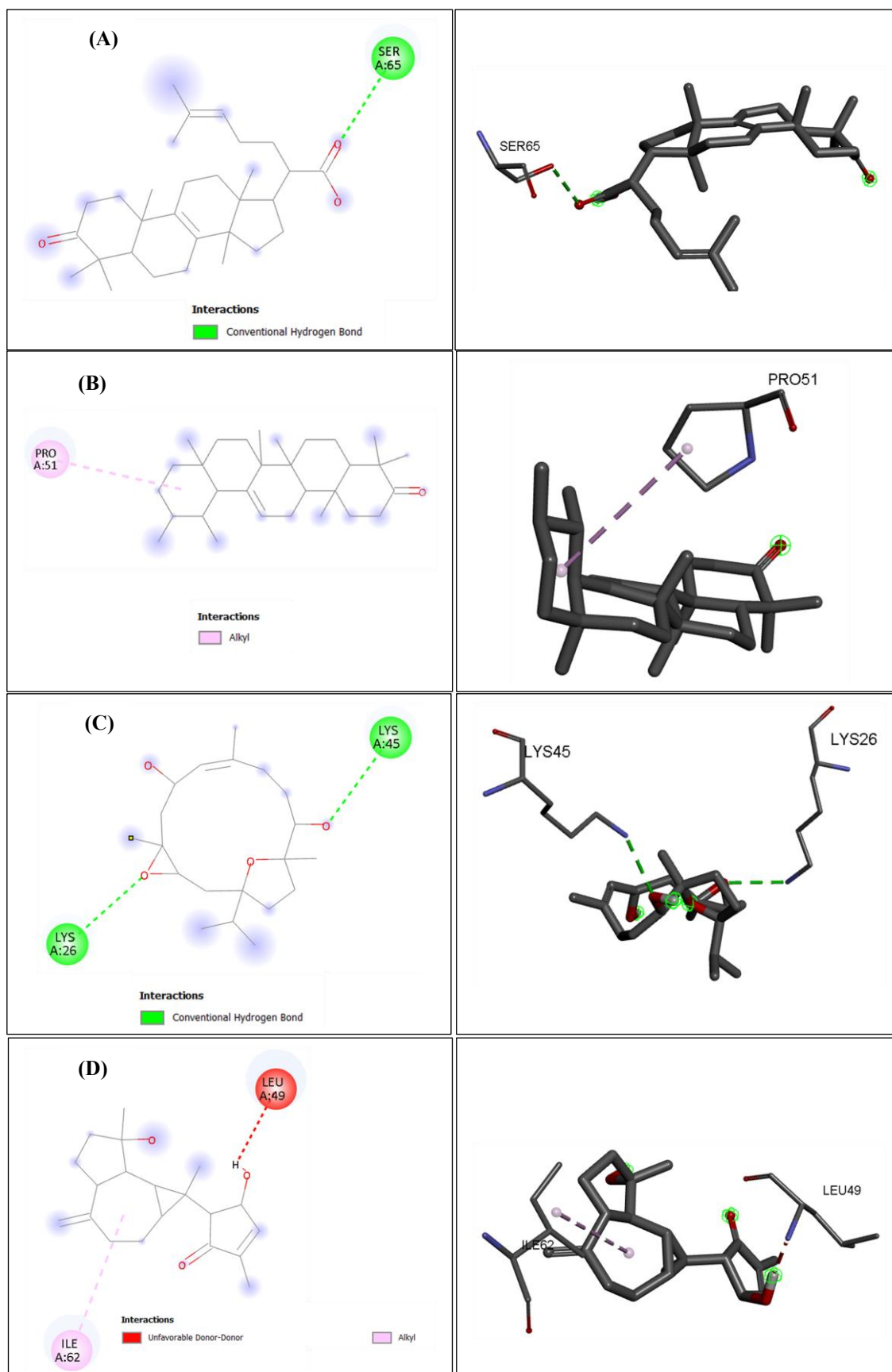
Suppl. Fig. 6. 2D and 3D pictures representing the Binding interactions of (A) Internal ligand, (B) 11-Keto boswellic acid, (C) 3-O-Acetyl 11-keto boswellic acid, and (D) 3-Acetyl-27-hydroxy-lupeolic acid in the active site of mTOR.



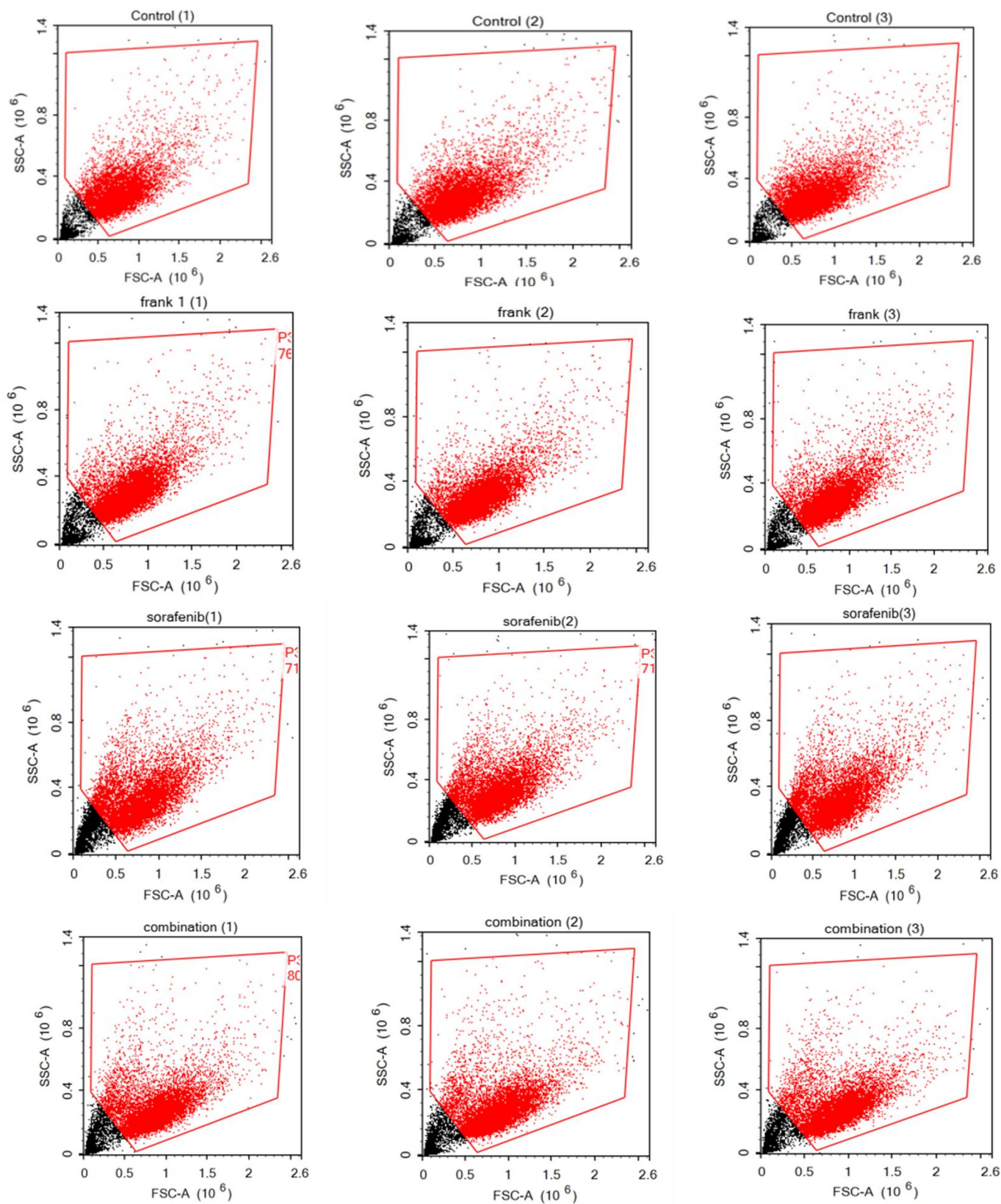
Suppl. Fig. 7. 2D and 3D pictures representing the Binding interactions of (A) Lupenone, (B) 3-oxo-tirucallic acid, (C) Boscartin C, and (D) Boscartol G in the active site of mTOR.



Suppl. Fig. 8. 2D and 3D pictures representing the Binding interactions of (A) Internal ligand, (B) 11-Keto boswellic acid, (C) Lupenone, and (D) 3-hydroxytirucallic acid in the active site of LC3C.

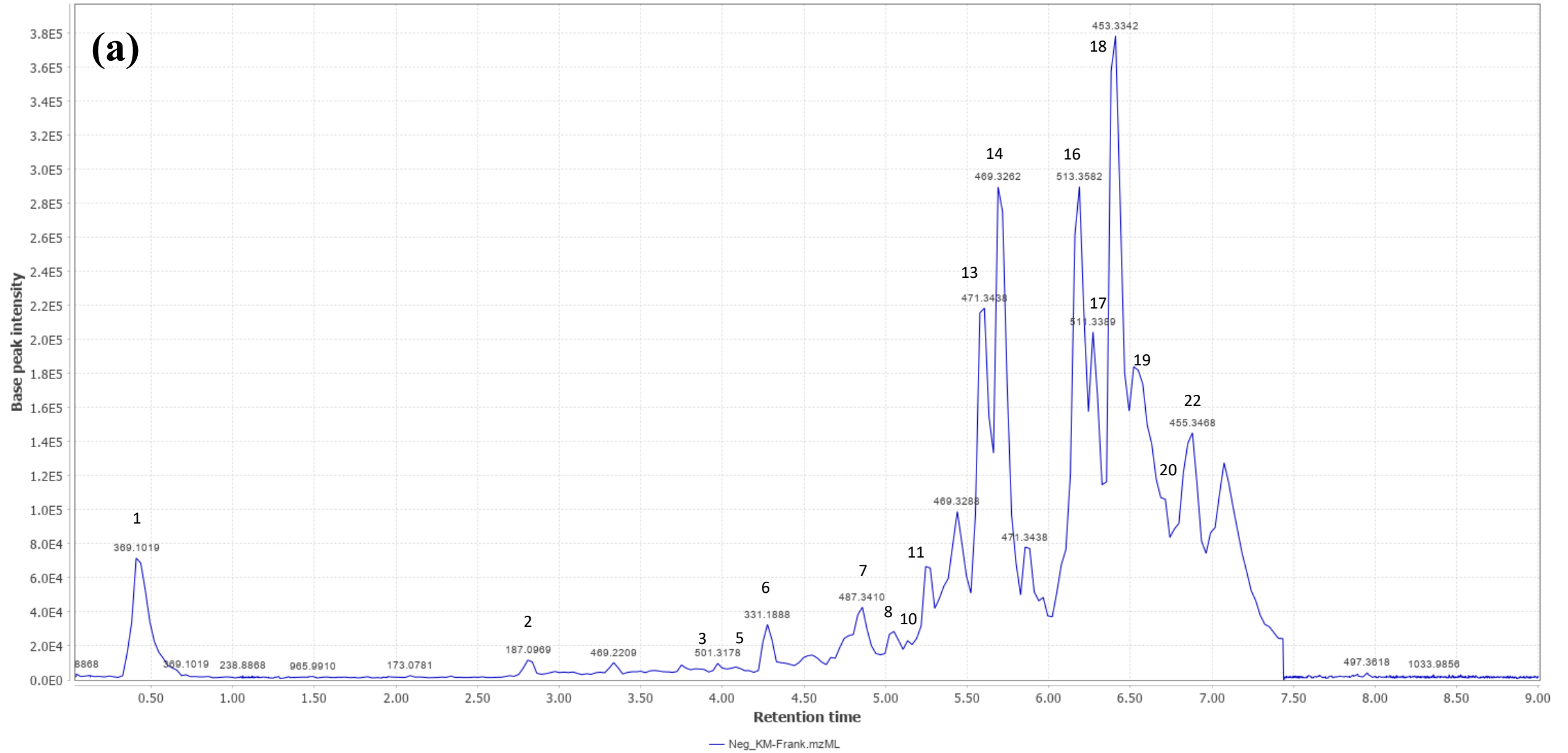


Suppl. Fig. 9. 2D and 3D pictures representing the Binding interactions of (A) 3-oxo-tirucallic acid, (B) α -Amyrenone, (C) Boscartin C, and (D) Boscartol G in the active site of LC3C.

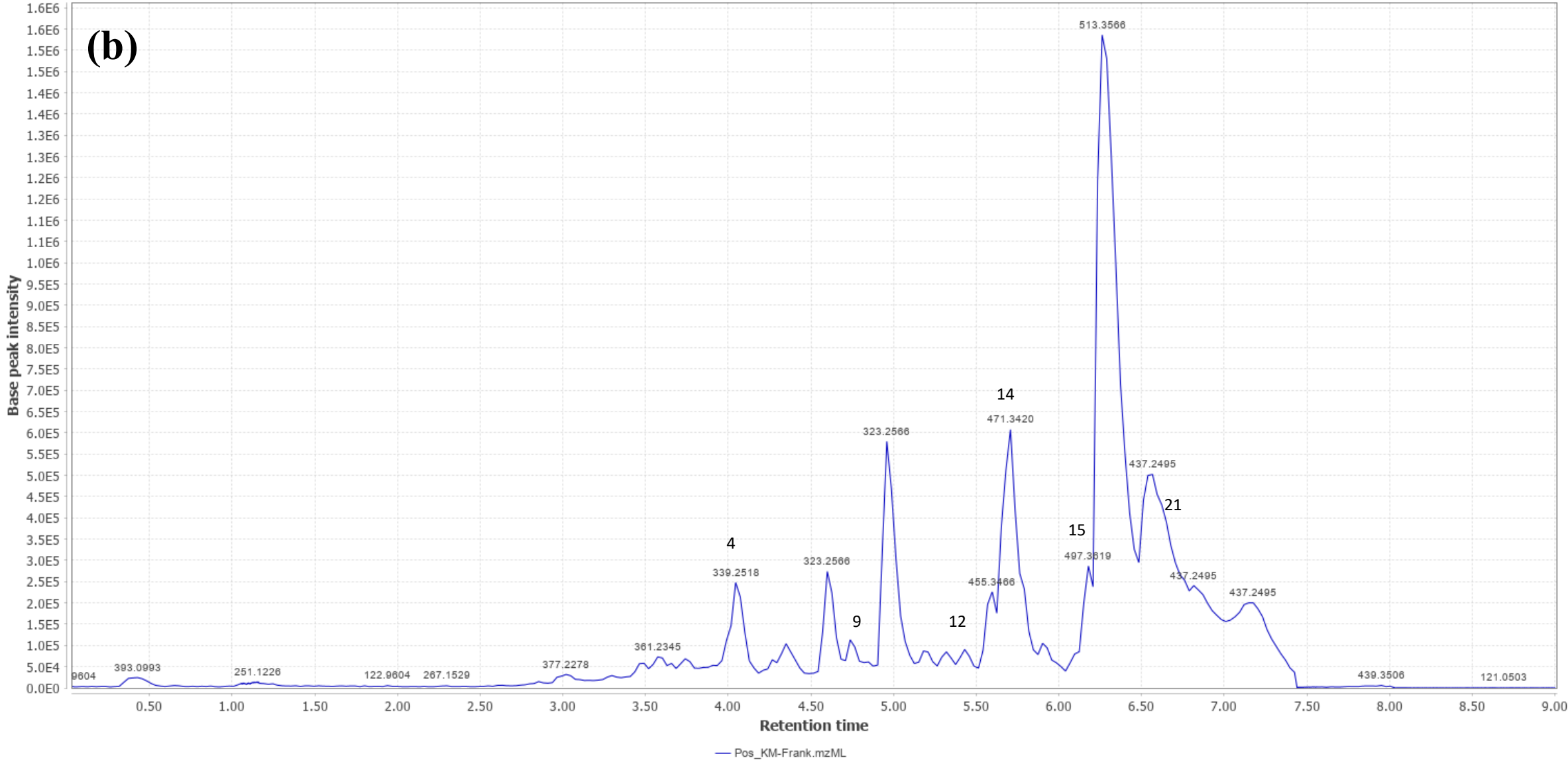


Suppl. Fig. 10. The raw apoptosis/necrosis assay data for Figure 12 demonstrate consistent gating across all tested experimental groups, for clarification and reliable verification of the results.

XIC (base peak), m/z: 99.9951 - 1999.0860

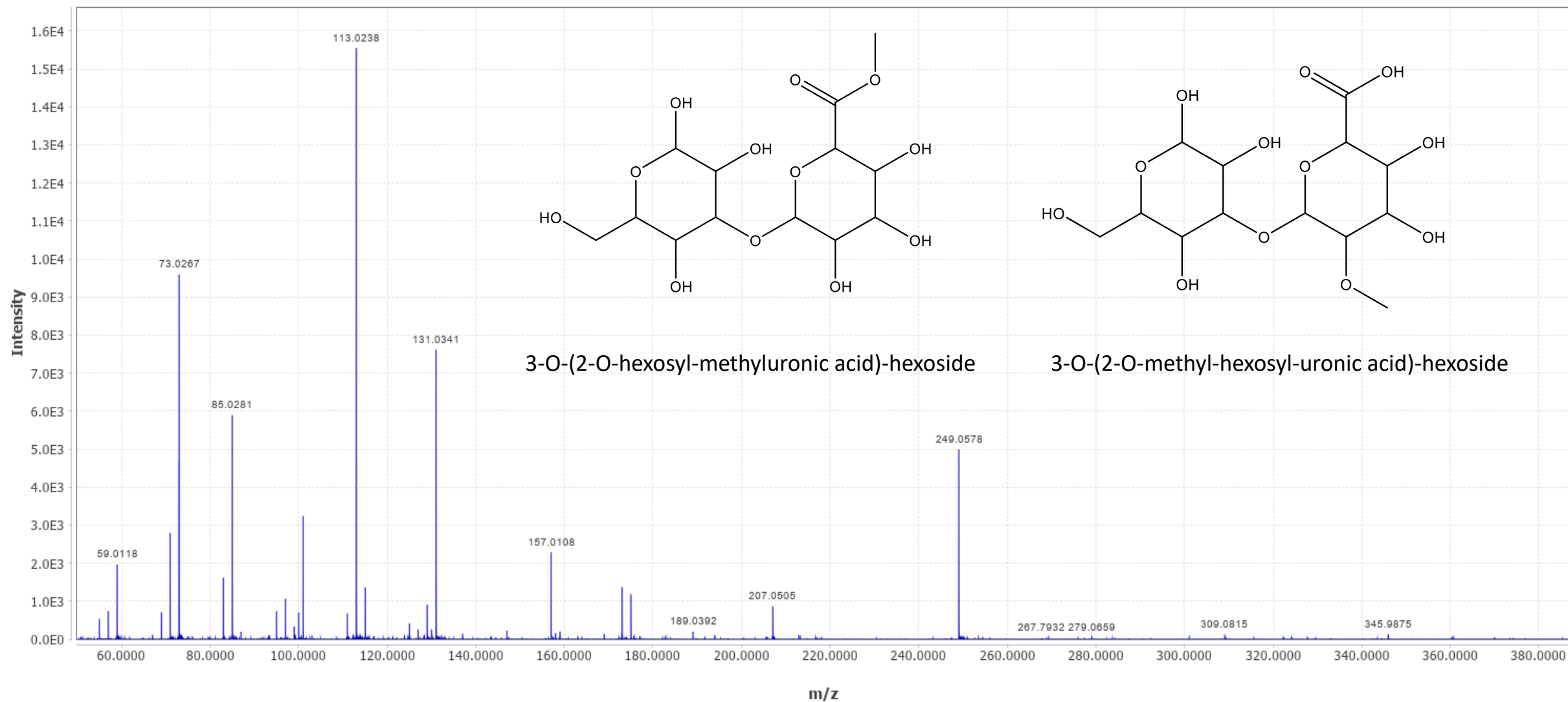


XIC (base peak), m/z: 99.9951 - 1999.0860



Neg_KM-Frank.mzML#140 @0.42 MS2 (369.1029) p -, base peak: 113.0238 m/z (1.6E4)

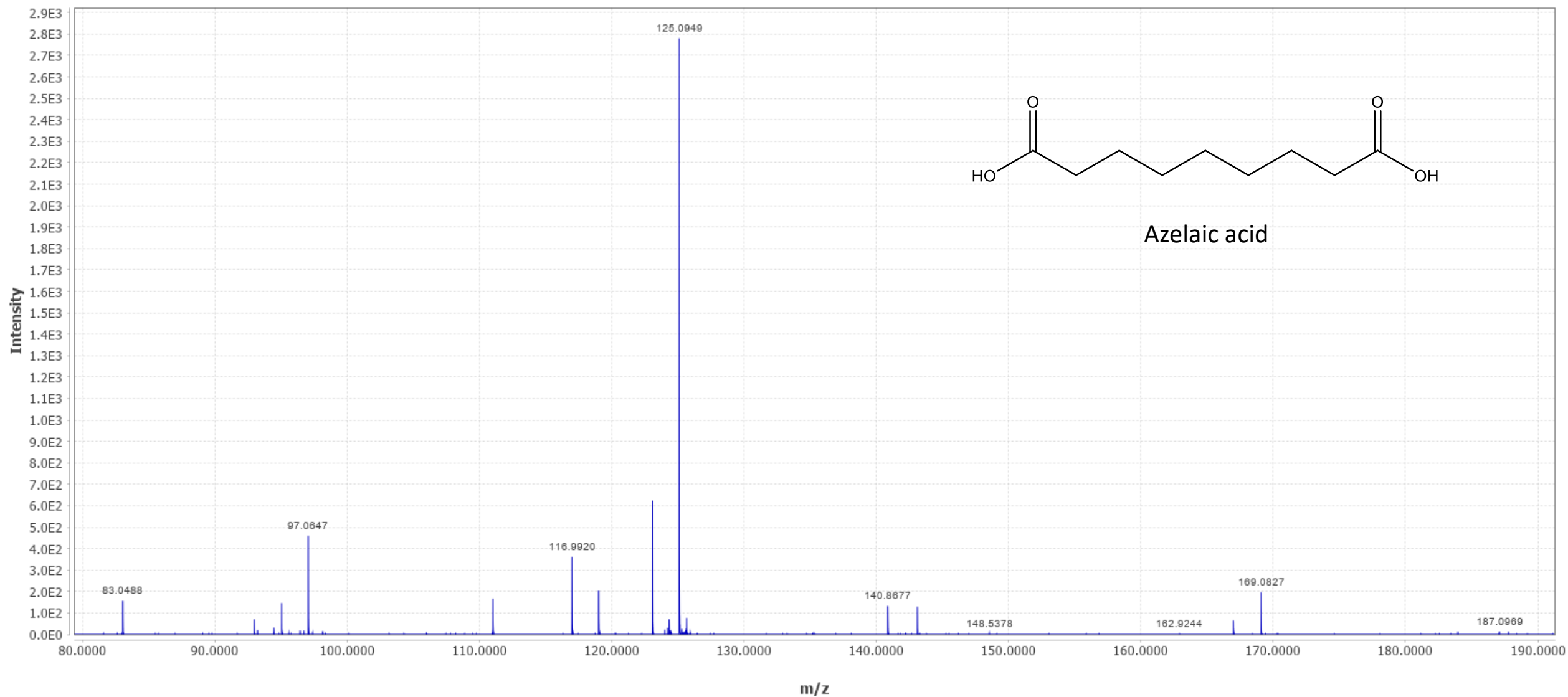
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— Scan #140 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#1029 @2.79 MS2 (187.0966) p -, base peak: 125.0949 m/z (2.8E3)

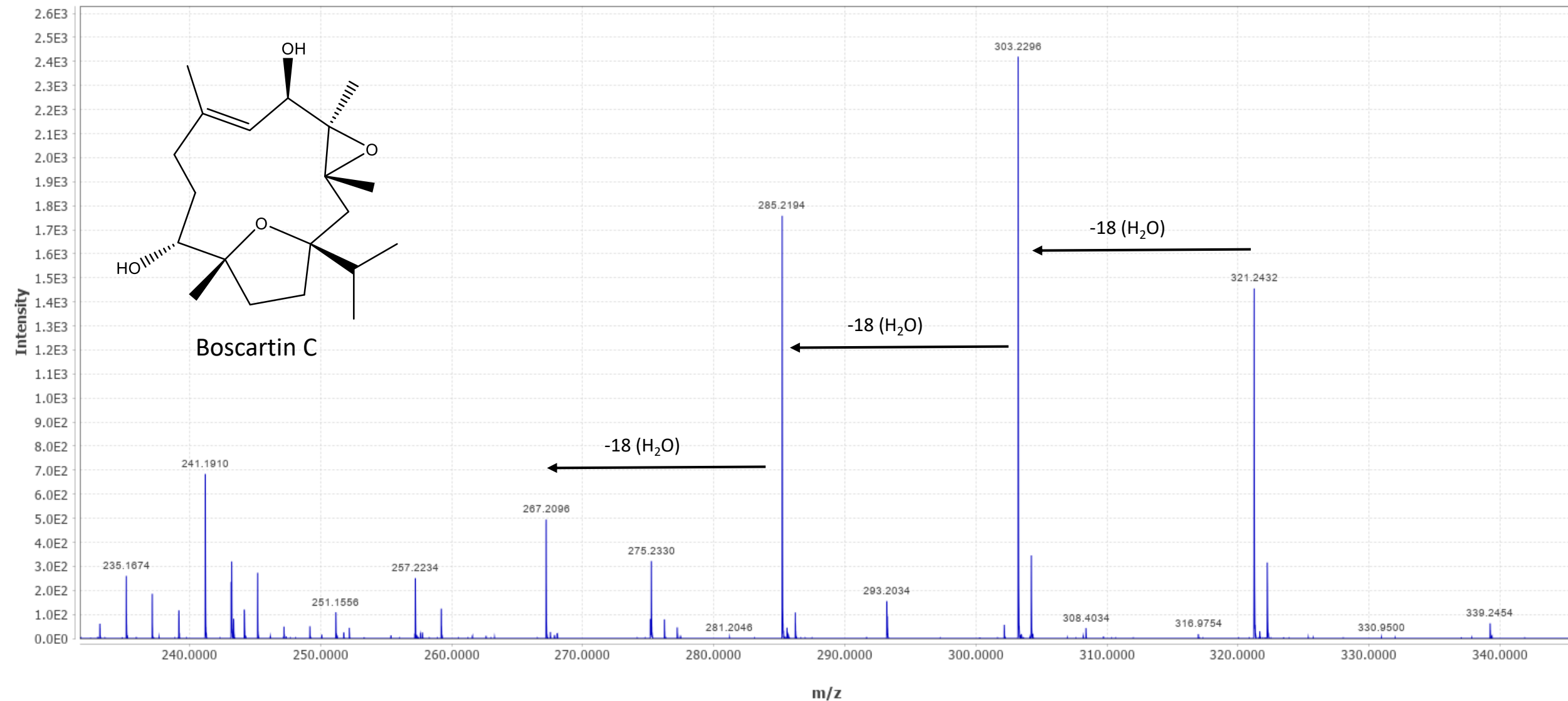
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— Scan #1029 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Pos_KM-Frank.mzML#1470 @4.03 MS2 (339.2527) p +, base peak: 151.1102 m/z (1.3E4)

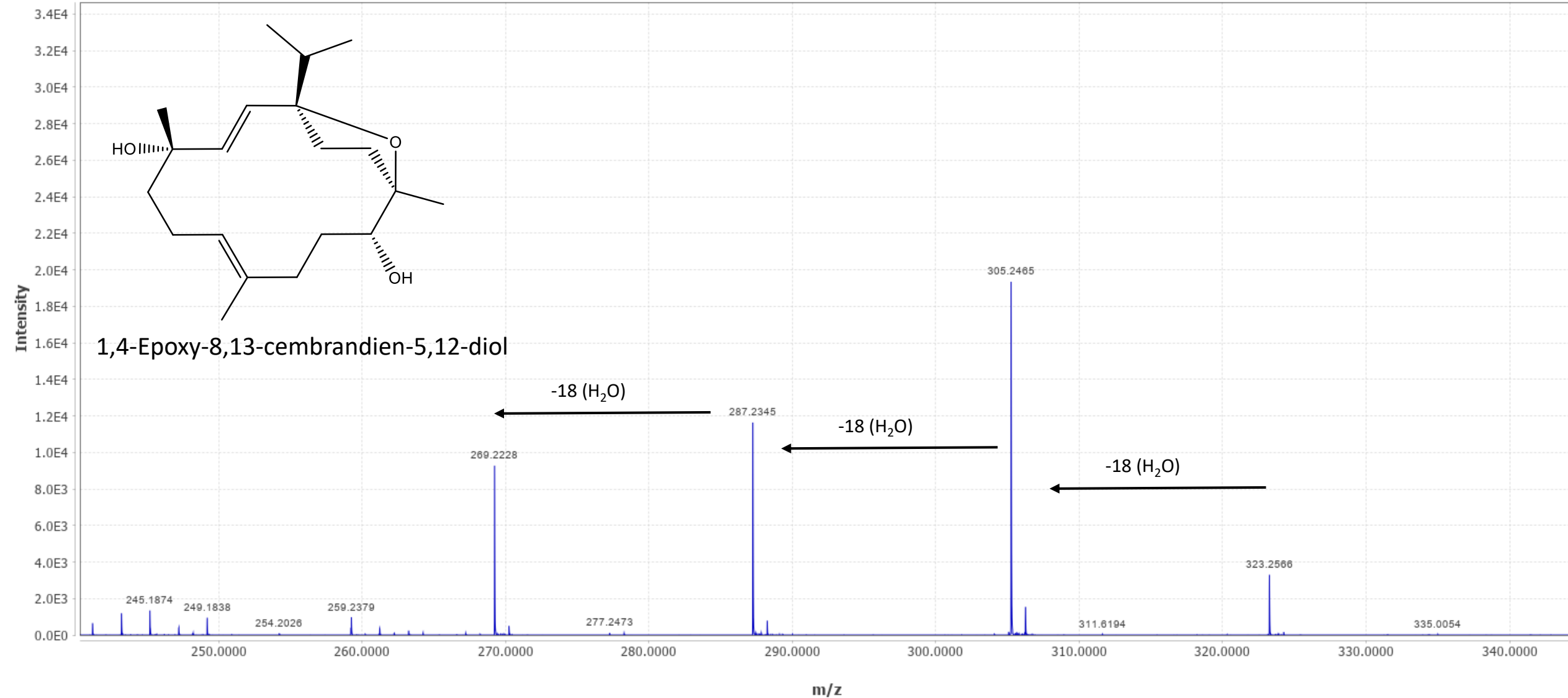
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— Scan #1470 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped

Pos_KM-Frank.mzML#1809 @4.97 MS2 (323.2583) p +, base peak: 123.1145 m/z (3.4E4)

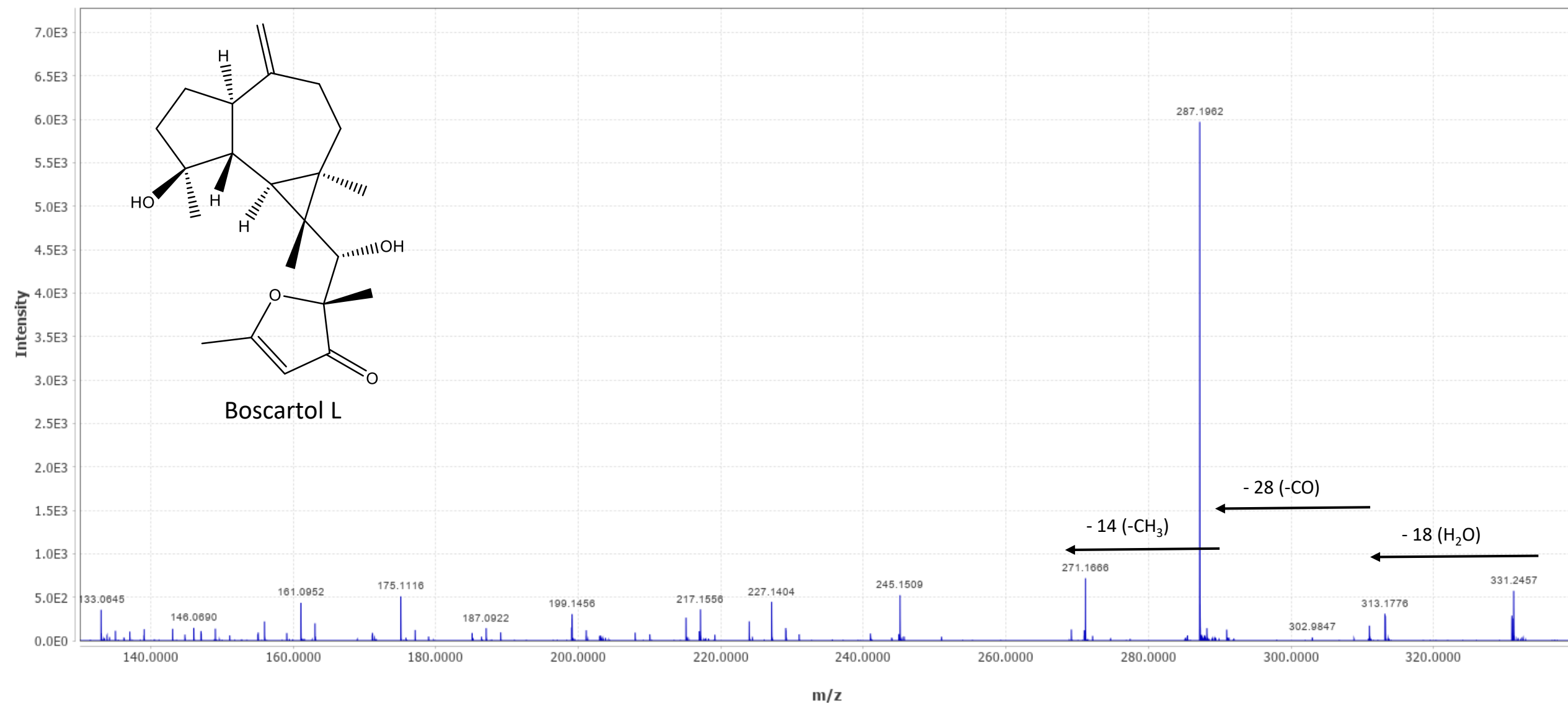
Scan definition: scanId=298073



— Scan #1809 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#1559 @4.26 MS2 (331.1905) p -, base peak: 287.1962 m/z (6.0E3)

Scan definition: scanId=255410

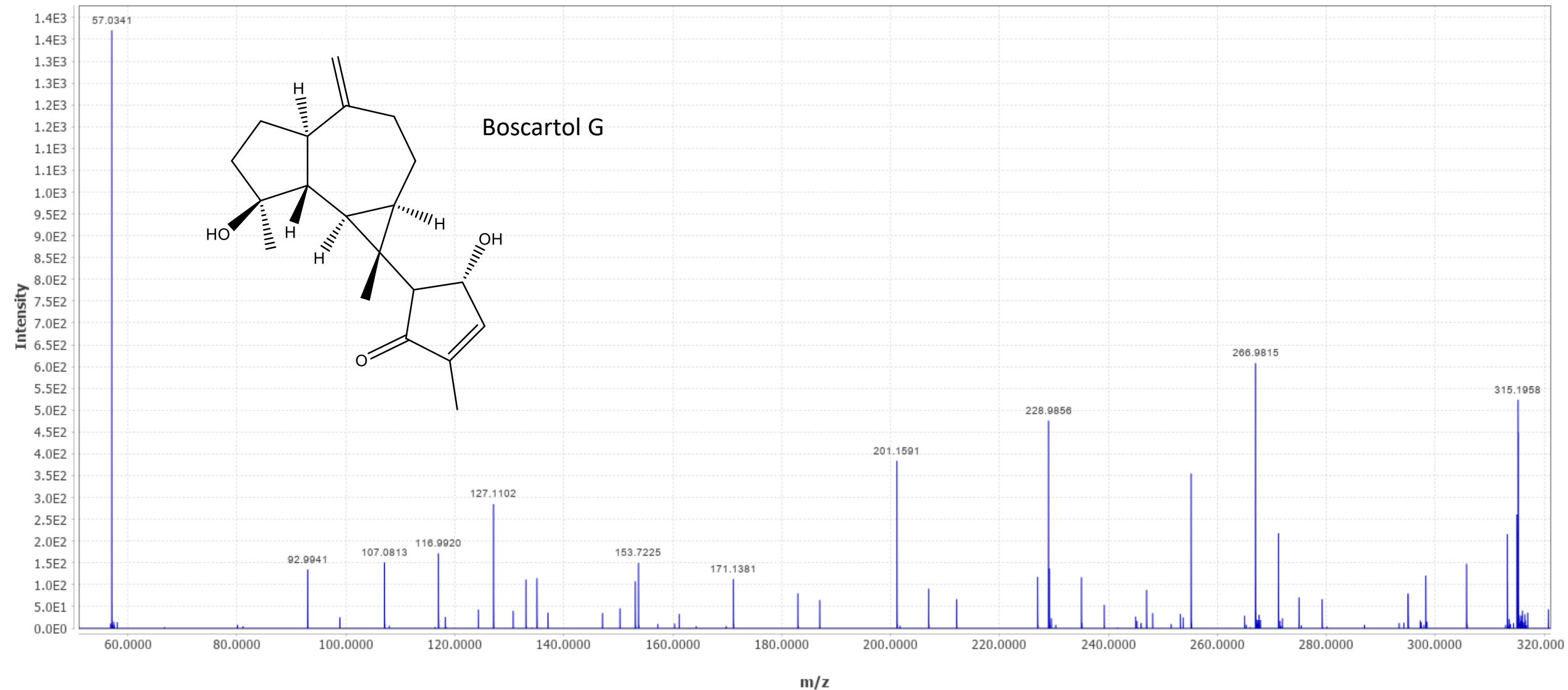
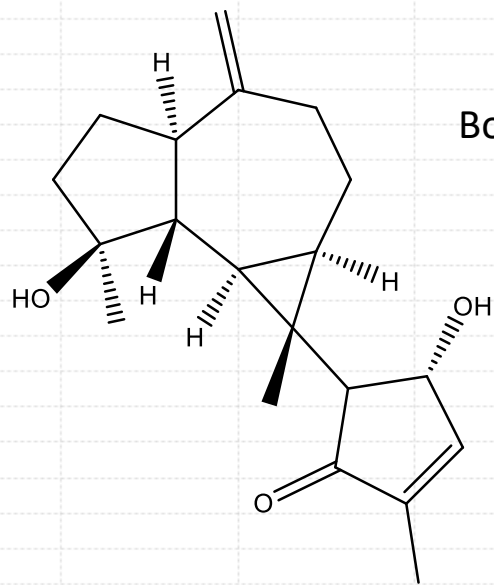


— Scan #1559 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#1781 @4.87 MS2 (315.1954) p-, base peak: 57.0341 m/z (1.4E3)

Scan definition: scanId=292313

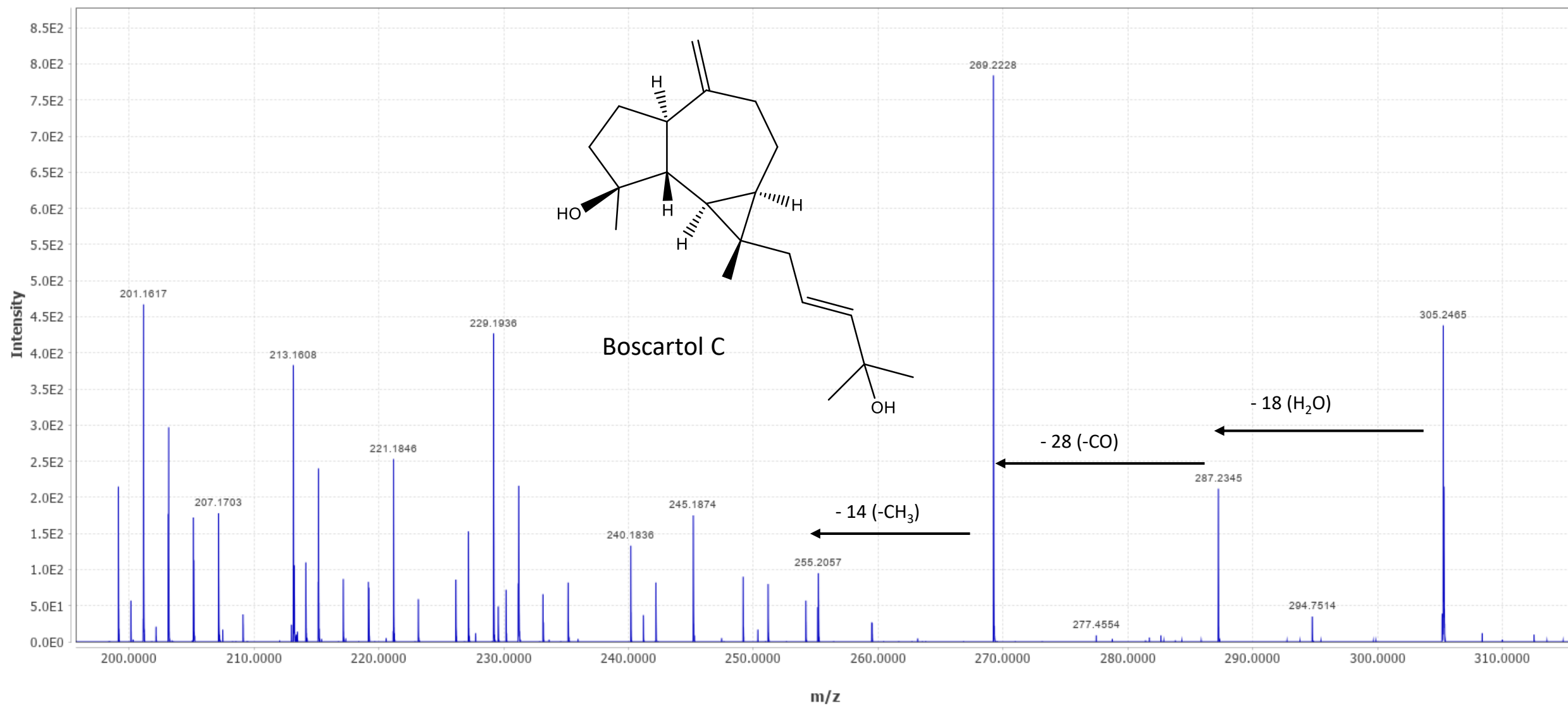
Boscartol G



Scan #1781

Pos_KM-Frank.mzML#2030 @5.58 MS2 (305.2476) p +, base peak: 121.1000 m/z (7.3E3)

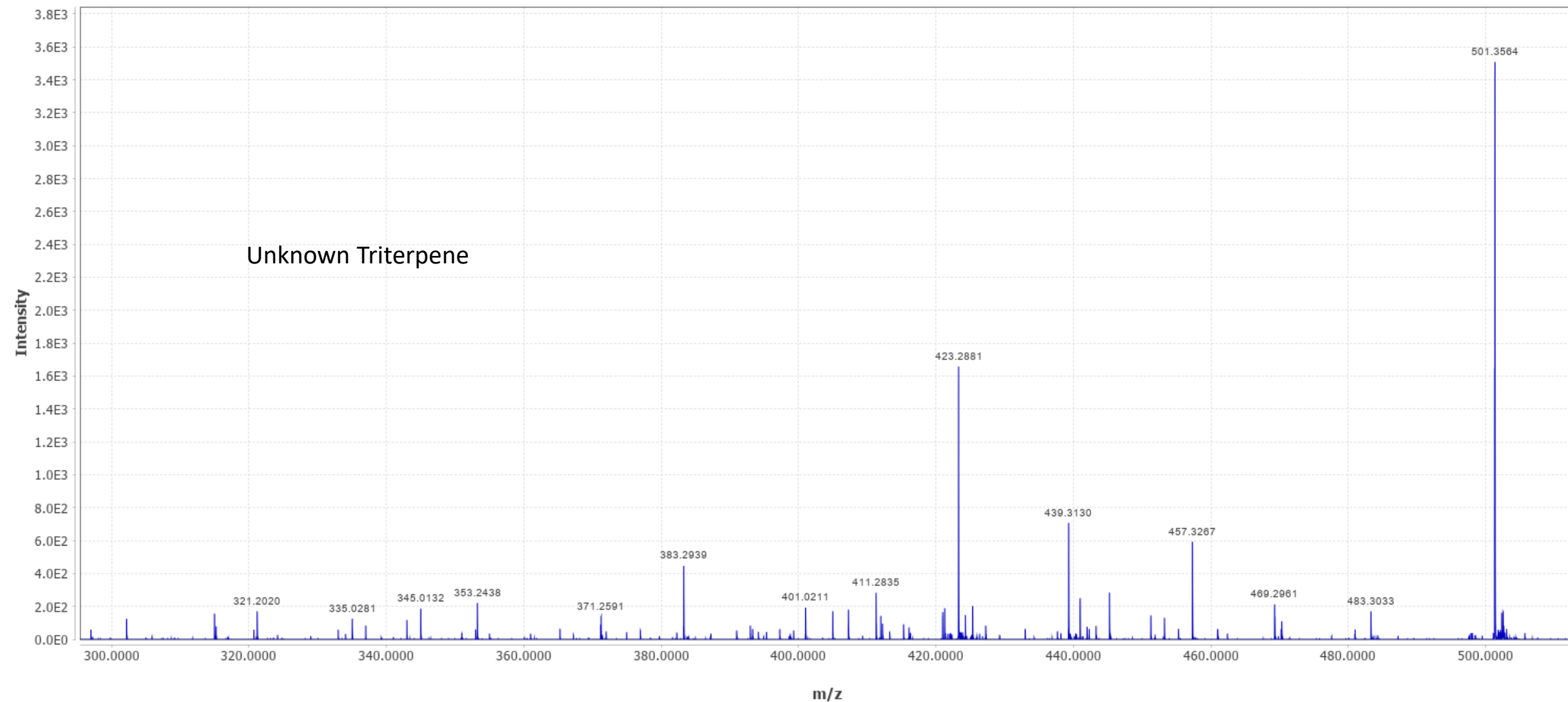
Scan definition: scanId=334808



— Scan #2030 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#1937 @5.30 MS2 (501.3219) p -, base peak: 501.3564 m/z (3.5E3)

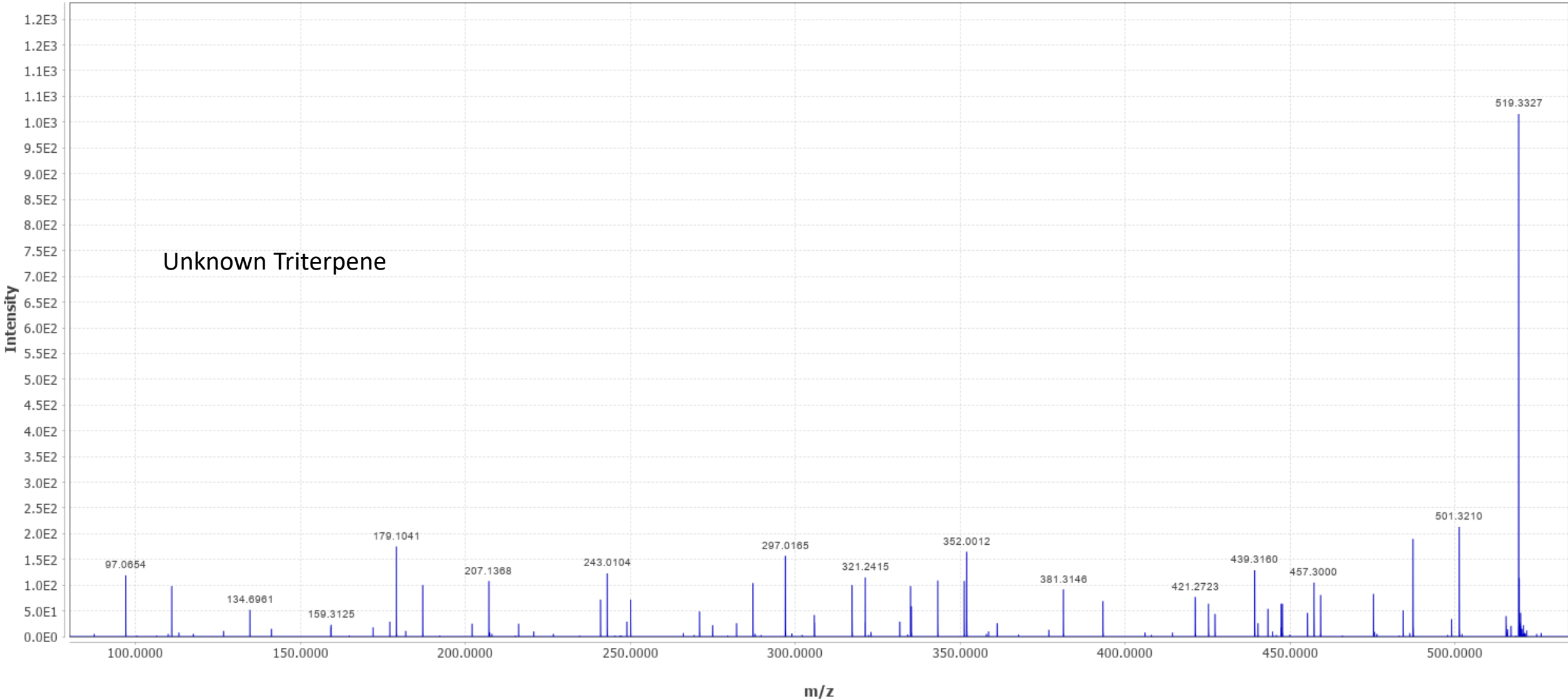
Scan definition: scanId=318250



— Scan #1937 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#1528 @4.17 MS2 (519.3309) p -, base peak: 519.3327 m/z (1.0E3)

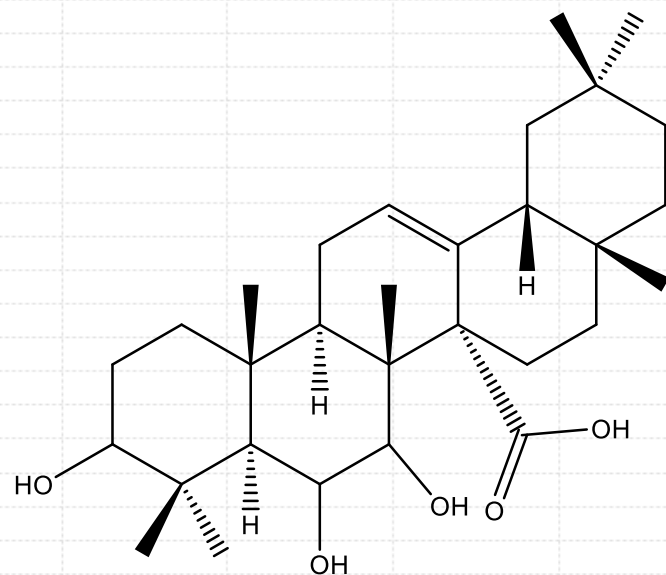
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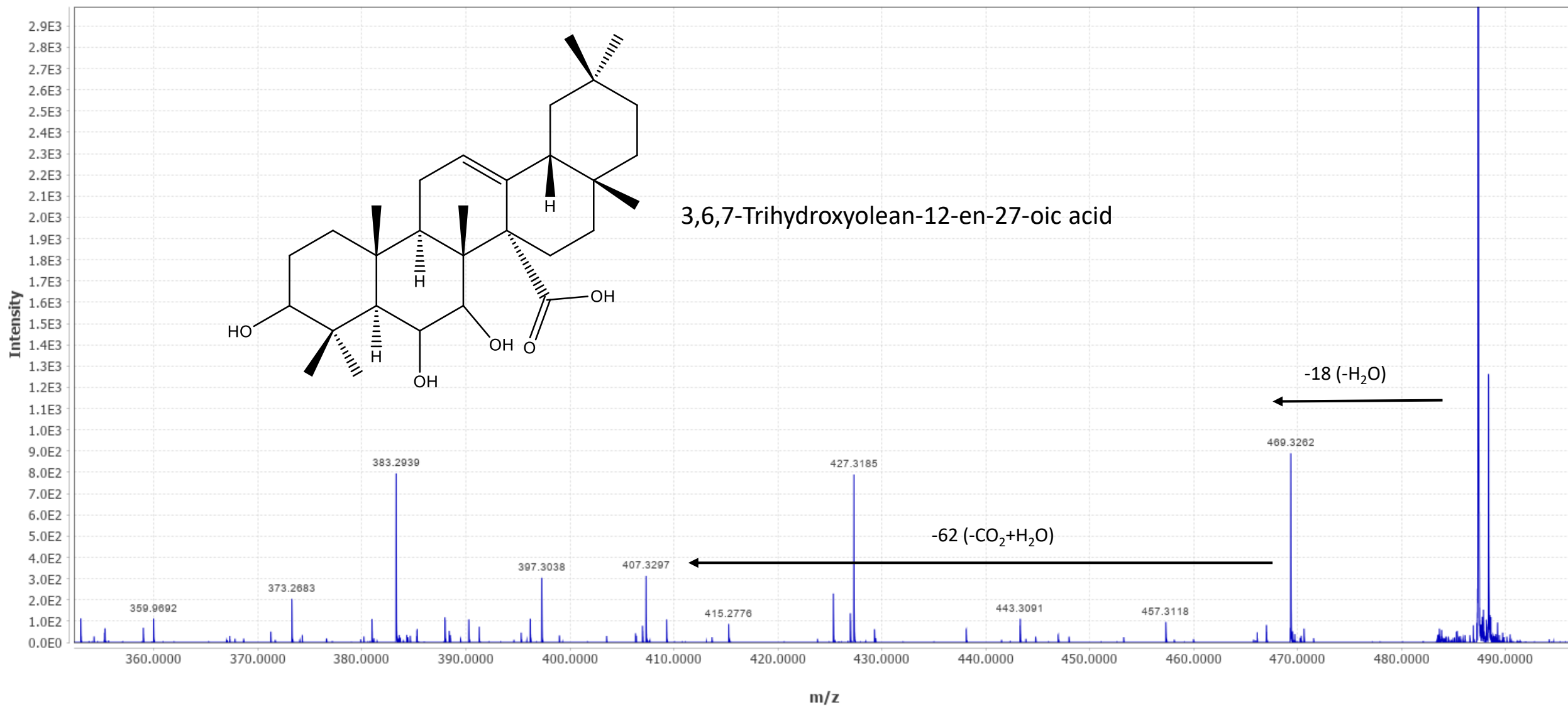
— Scan #1528 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#1759 @4.81 MS2 (487.3414) p -, base peak: 487.3410 m/z (2.1E4)

Scan definition: scanId=288658



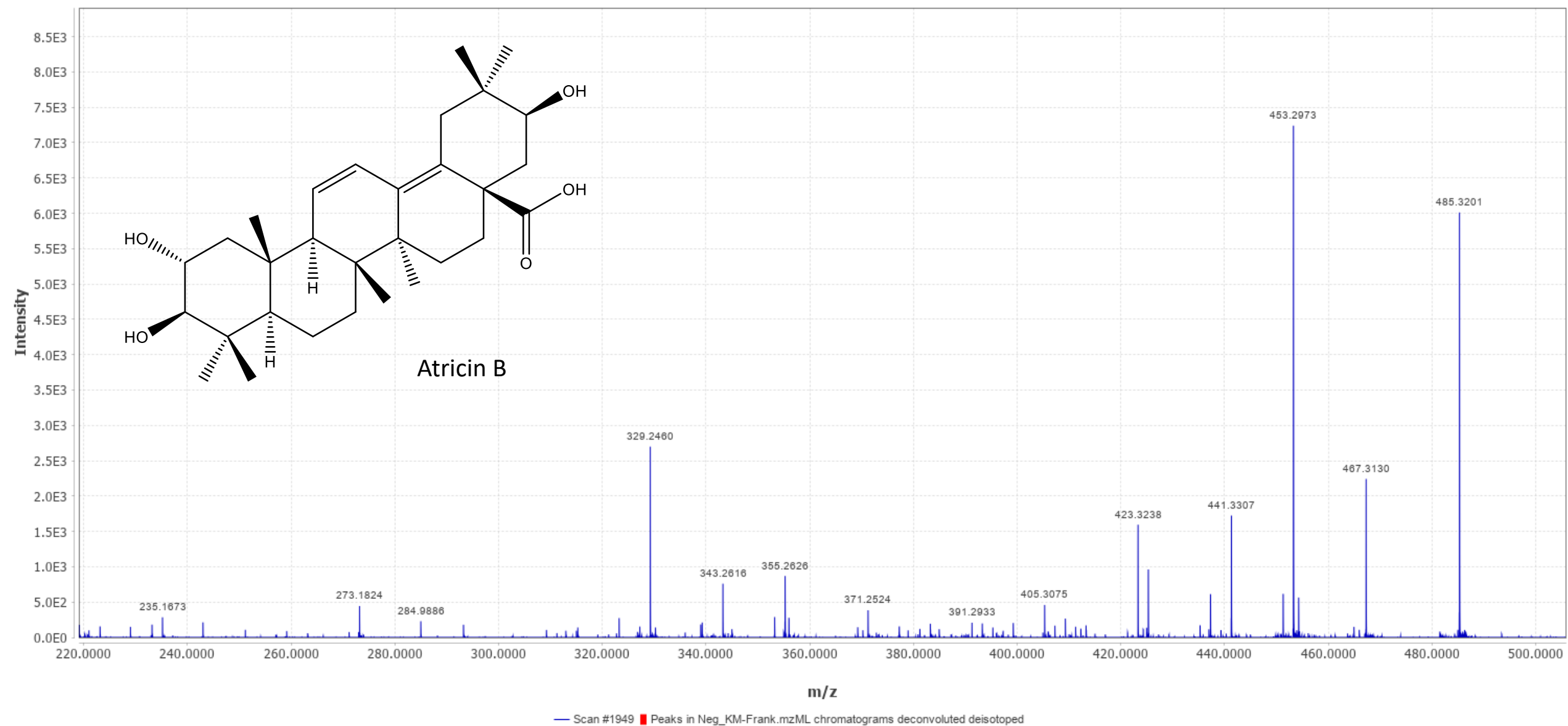
3,6,7-Trihydroxyolean-12-en-27-oic acid



— Scan #1759 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

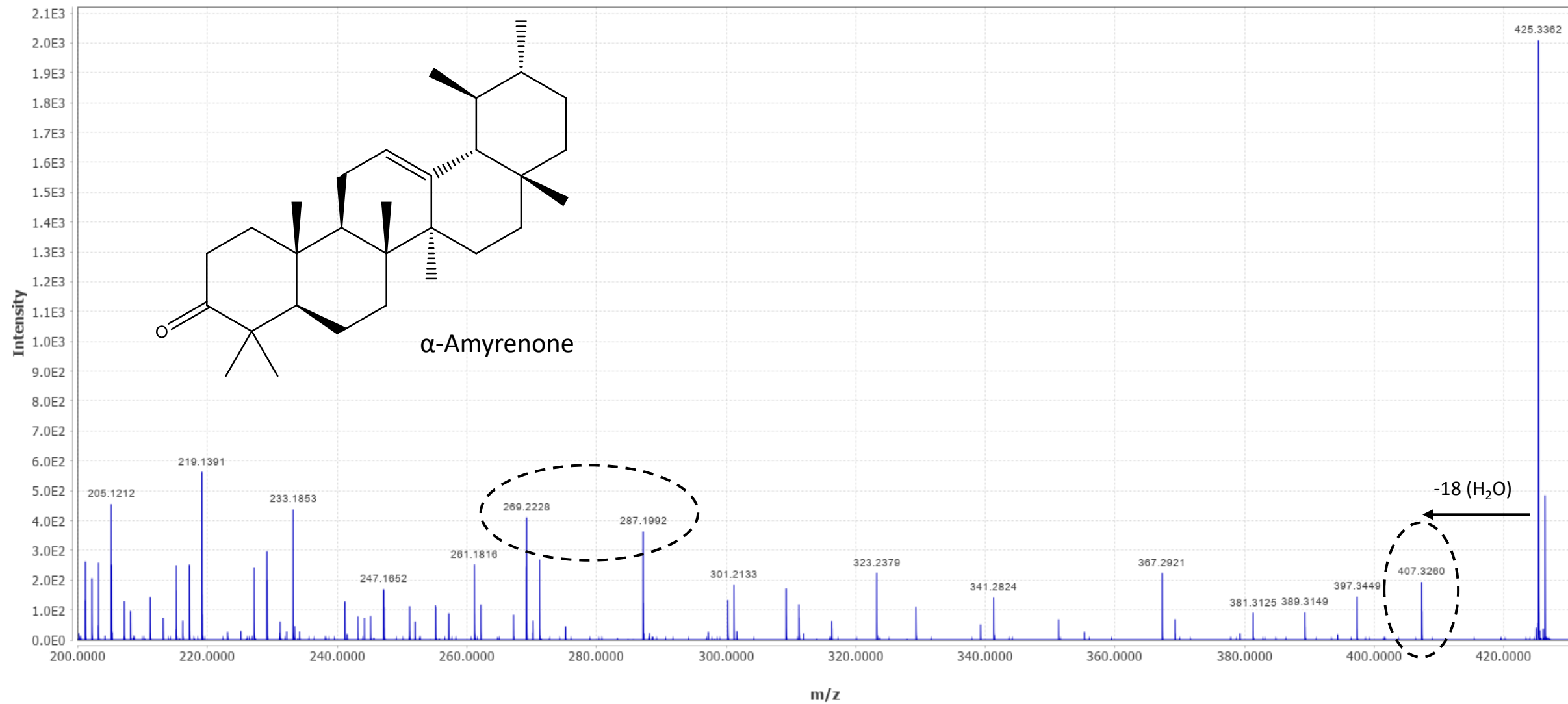
Neg_KM-Frank.mzML#1949 @5.34 MS2 (485.3263) p -, base peak: 453.2973 m/z (7.2E3)

Scan definition: scanId=320243



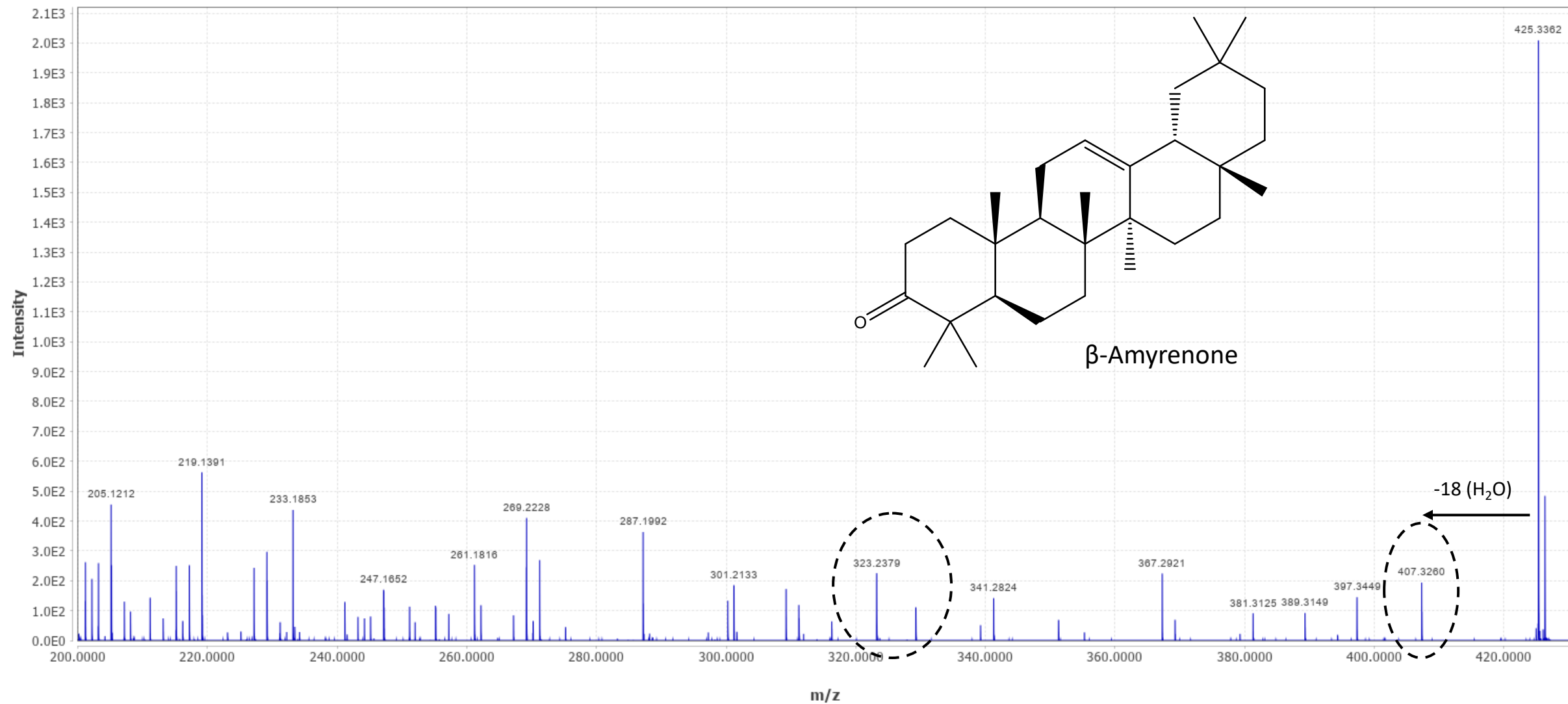
Pos_KM-Frank.mzML#2458 @6.77 MS2 (425.3772) p +, base peak: 95.0844 m/z (2.1E3)

Scan definition: scanId=405958

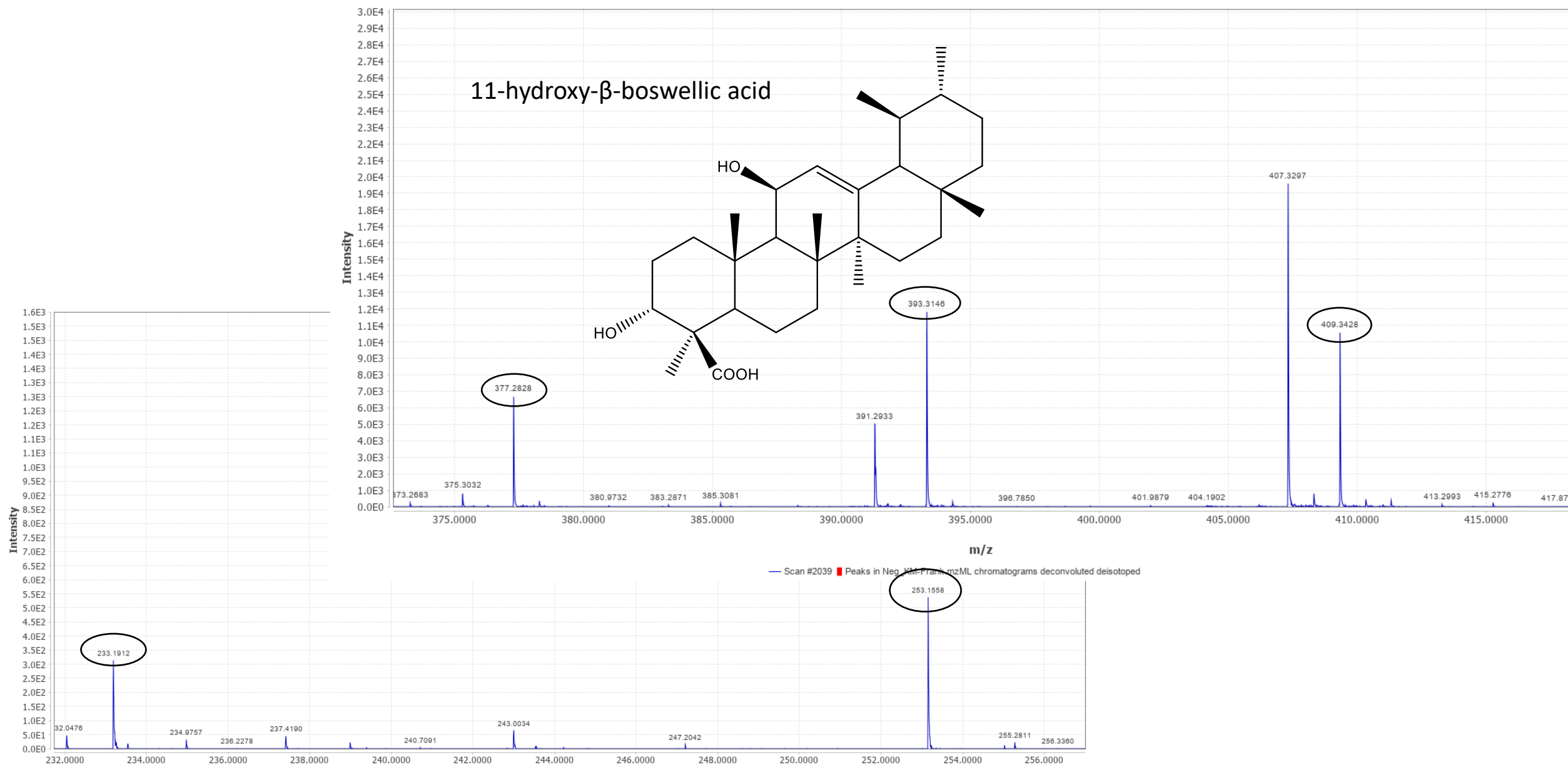


Pos_KM-Frank.mzML#2458 @6.77 MS2 (425.3772) p +, base peak: 95.0844 m/z (2.1E3)

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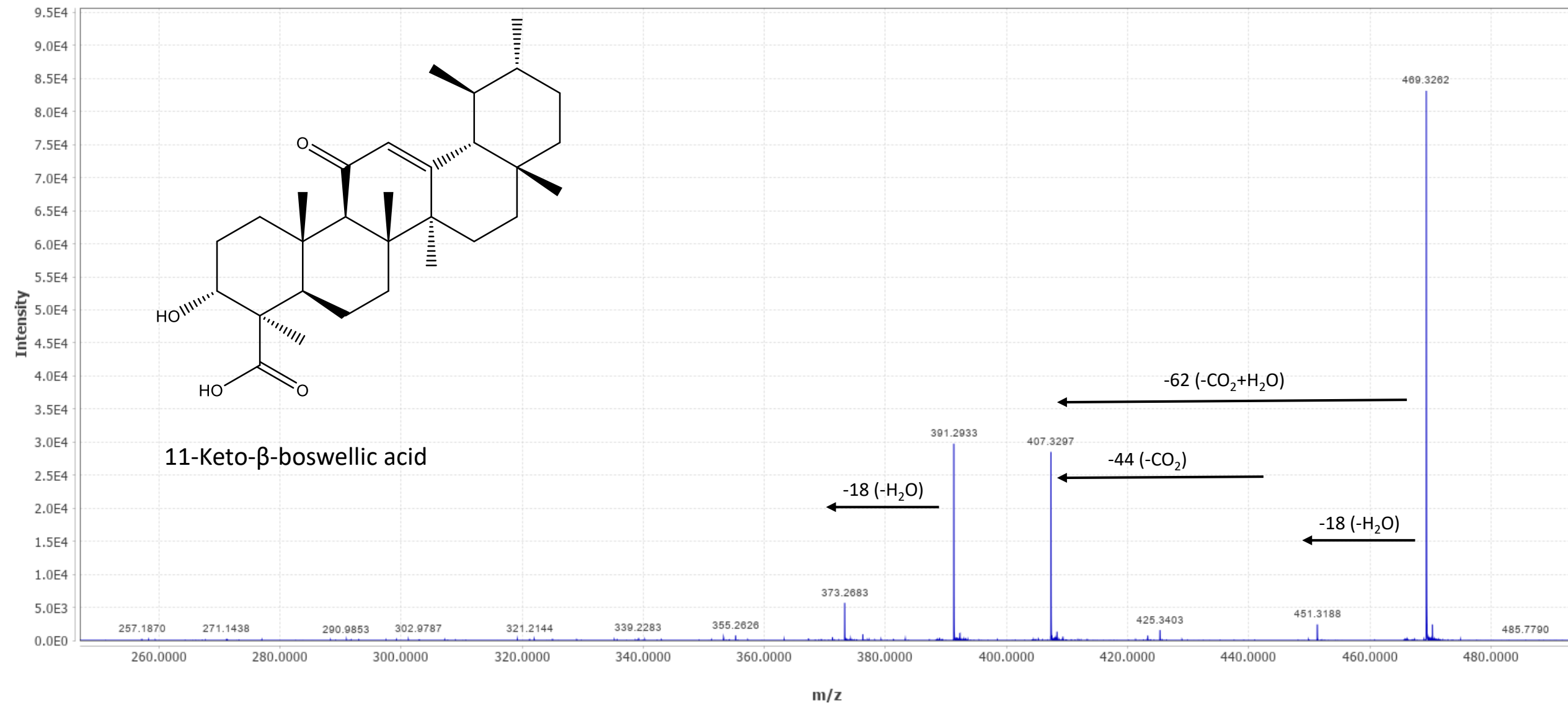


— Scan #2458 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped



Neg_KM-Frank.mzML#2089 @5.73 MS2 (469.3314) p -, base peak: 469.3262 m/z (8.3E4)

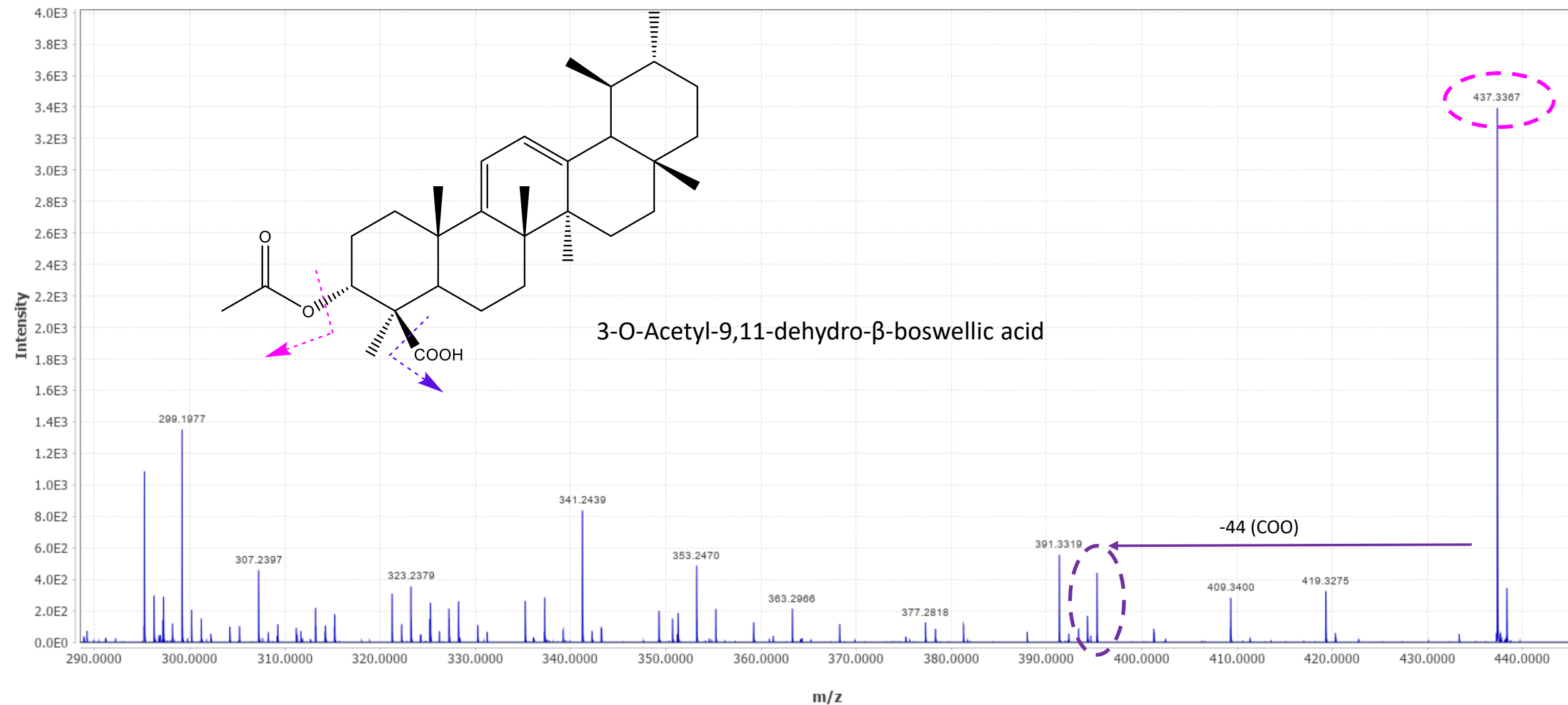
Scan definition: scanId=343517



— Scan #2089 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Pos_KM-Frank.mzML#2239 @6.16 MS2 (497.3629) p +, base peak: 95.0844 m/z (1.2E4)

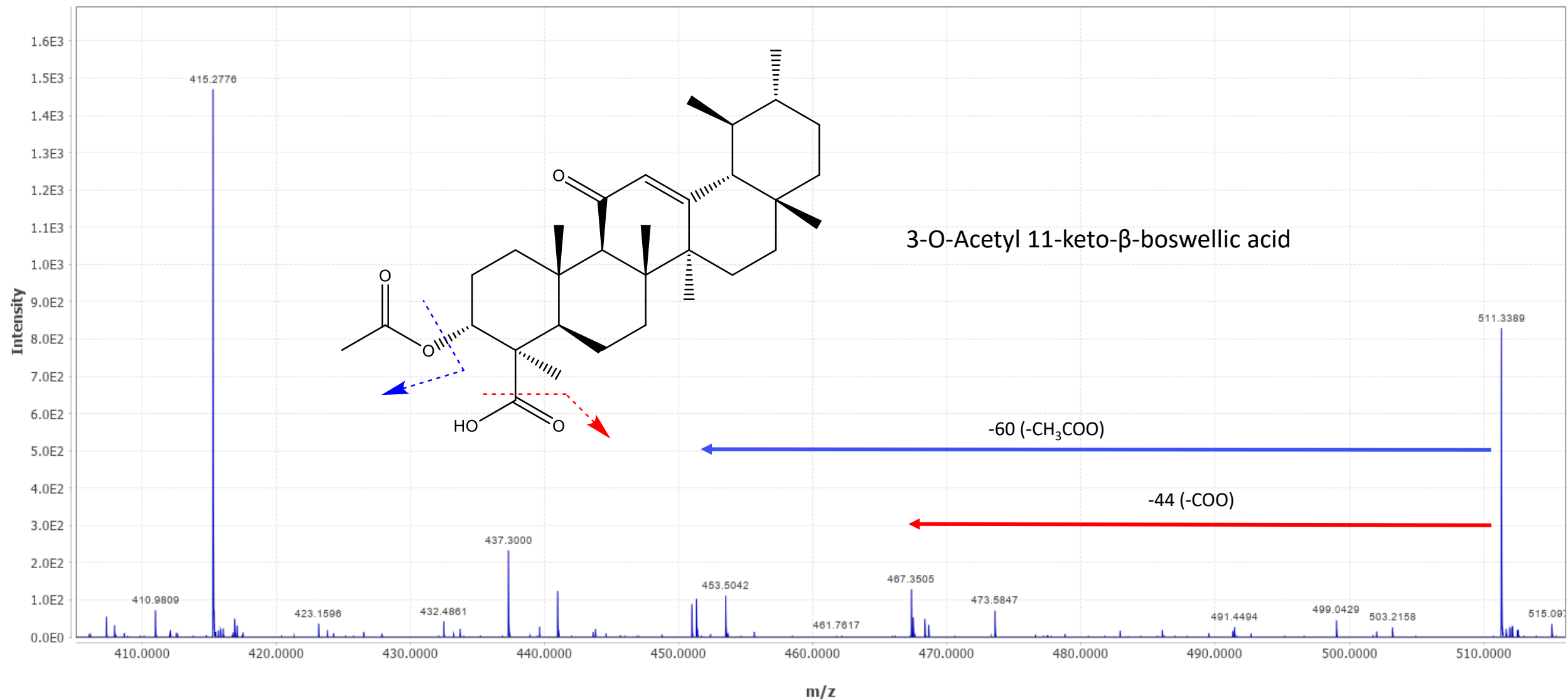
Scan definition: scanId=369552



— Scan #2239 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#2289 @6.28 MS2 (511.3416) p -, base peak: 59.0118 m/z (1.3E5)

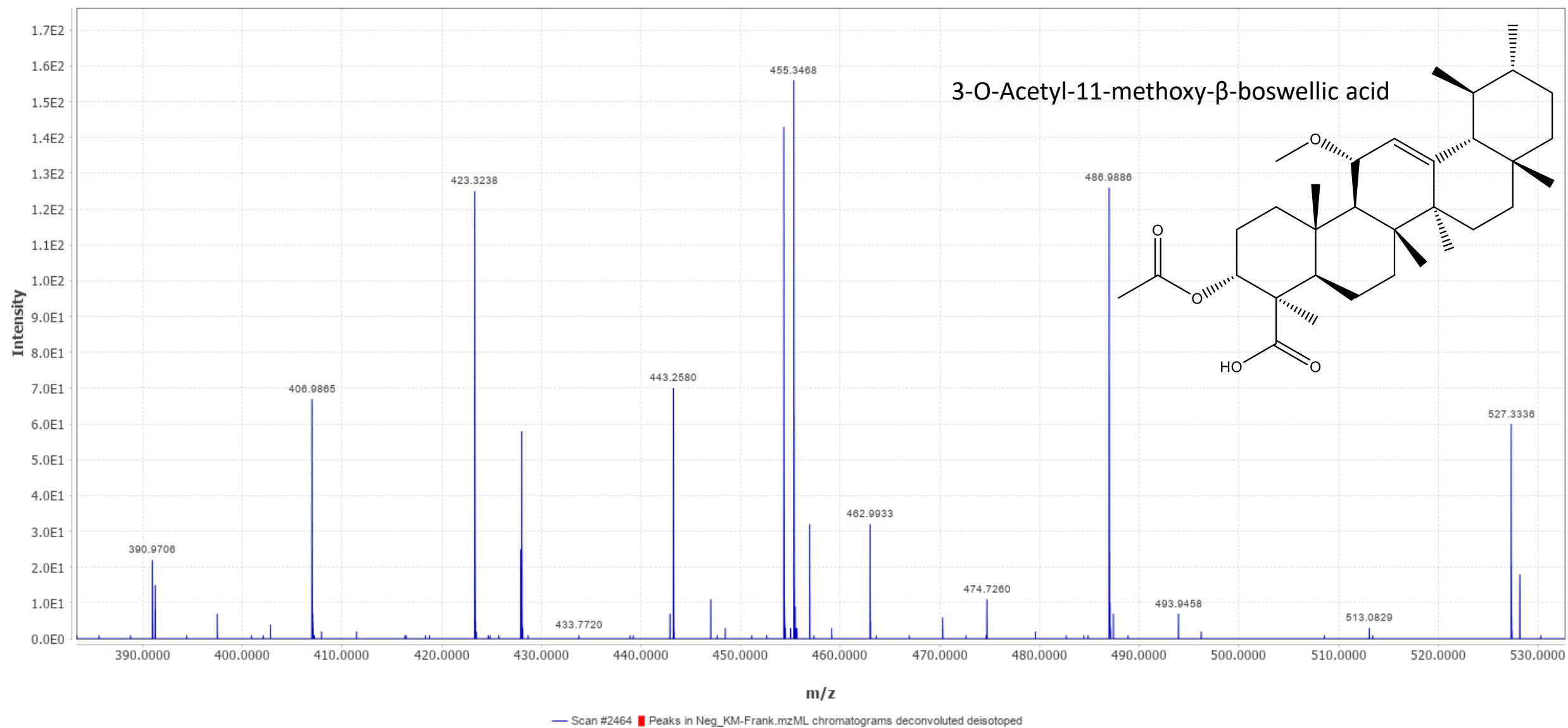
Scan definition: scanId=376766



— Scan #2289 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

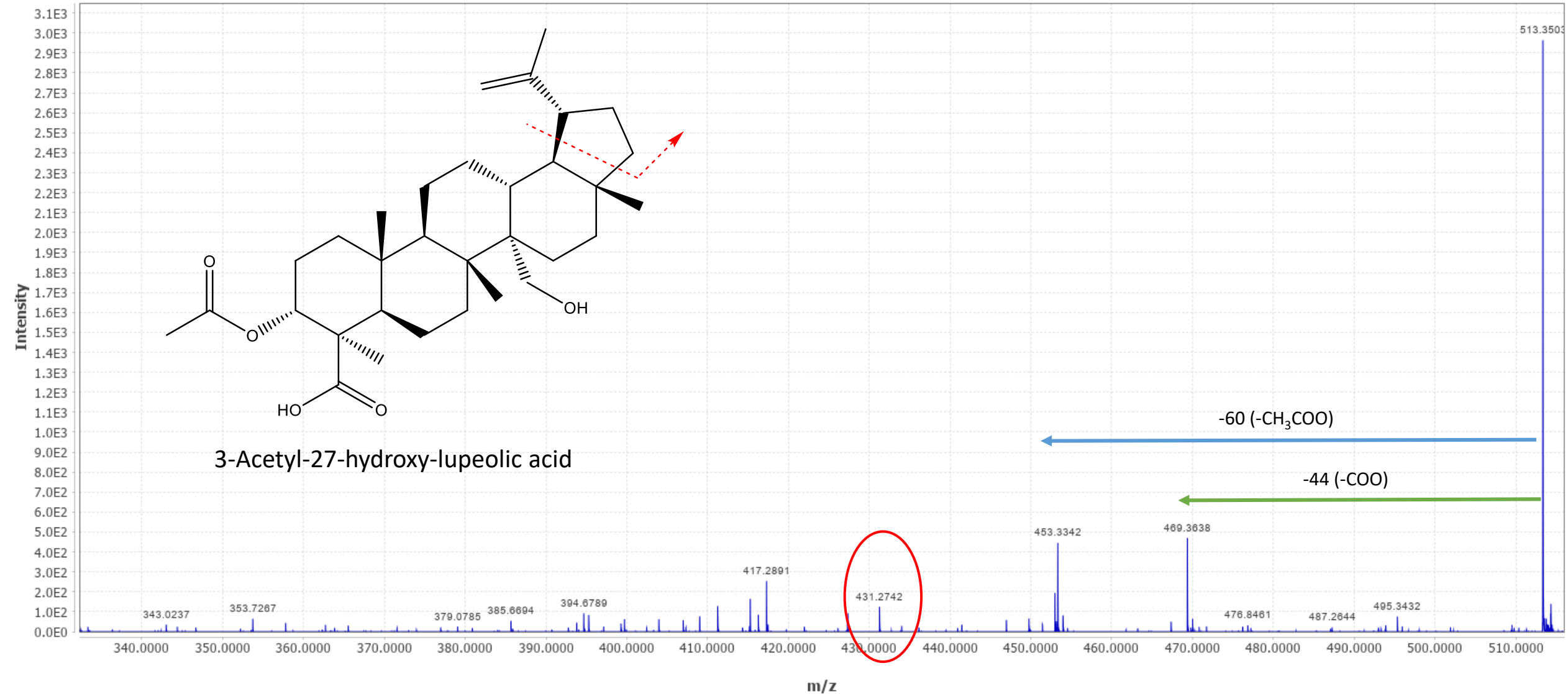
Neg_KM-Frank.mzML#2464 @6.76 MS2 (527.3727) p -, base peak: 59.0118 m/z (7.6E3)

Scan definition: scanId=405855



Neg_KM-Frank.mzML#2259 @6.20 MS2 (513.3572) p -, base peak: 59.0118 m/z (1.8E5)

Scan definition: scanId=371778

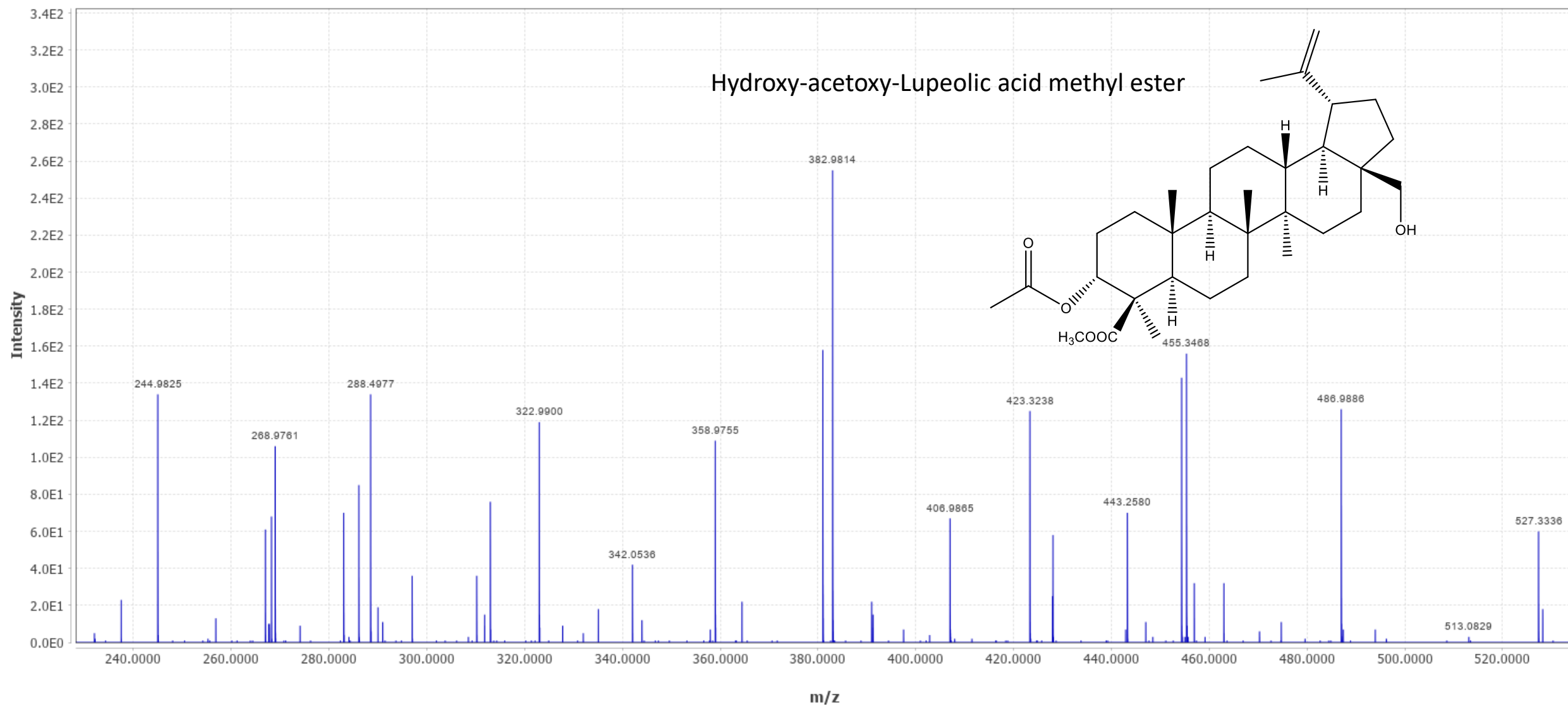
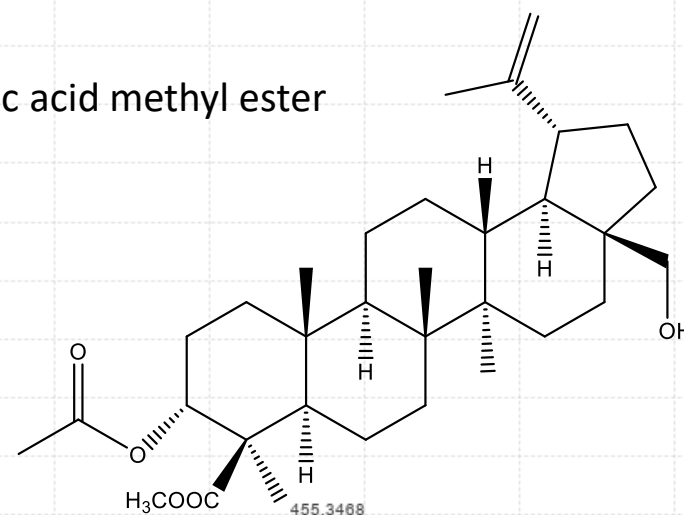


— Scan #2259 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

Neg_KM-Frank.mzML#2464 @6.76 MS2 (527.3727) p -, base peak: 59.0118 m/z (7.6E3)

Scan definition: scanId=405855

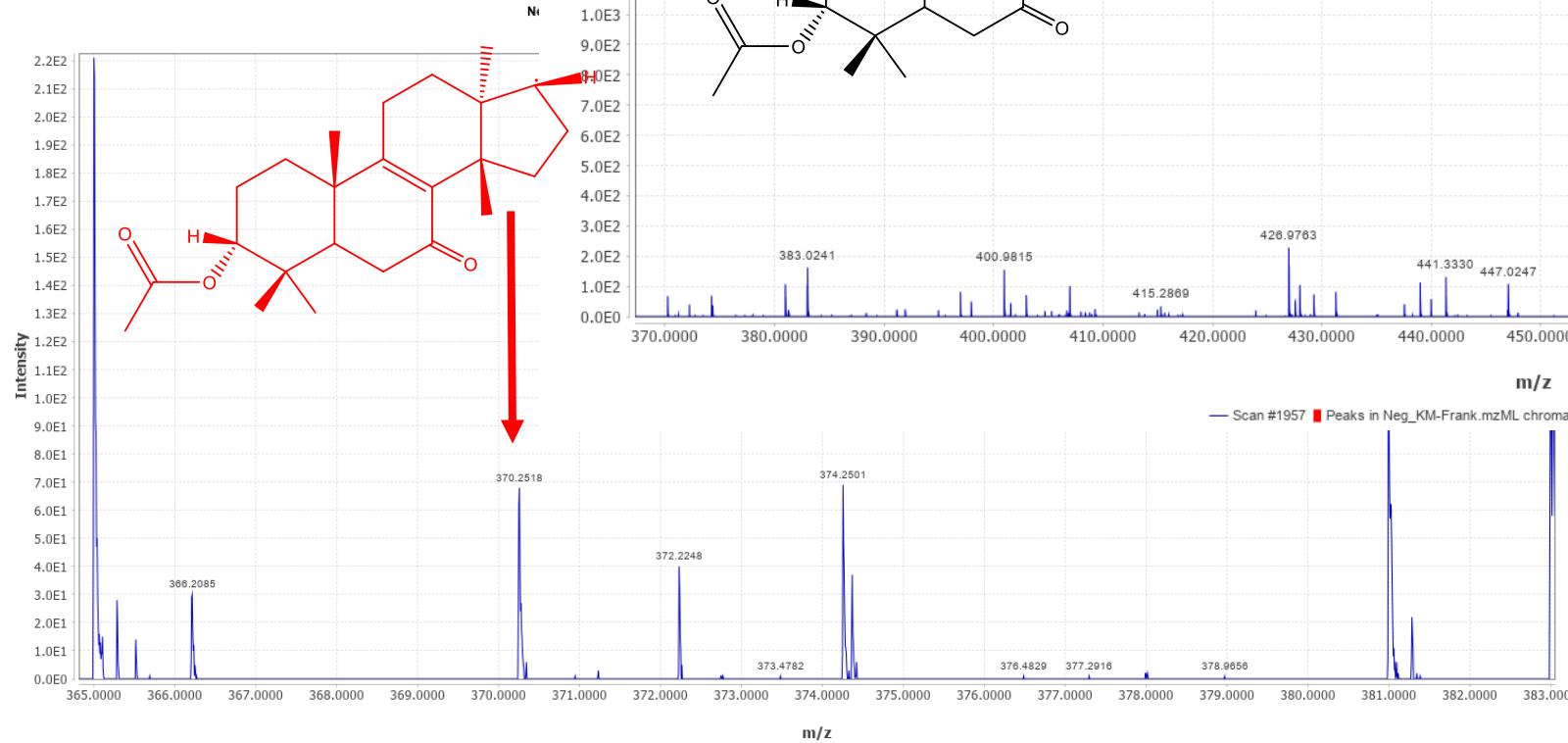
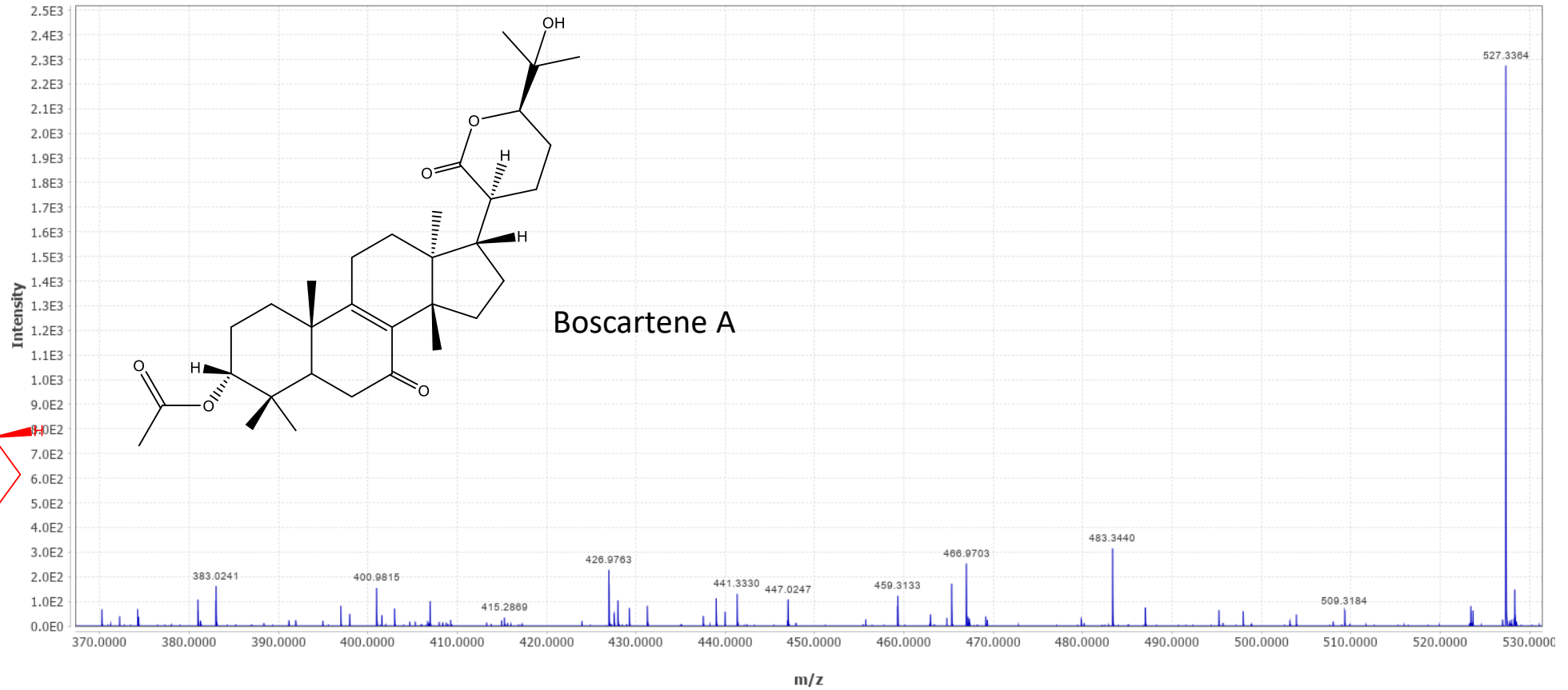
Hydroxy-acetoxy-Lupeolic acid methyl ester



— Scan #2464 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

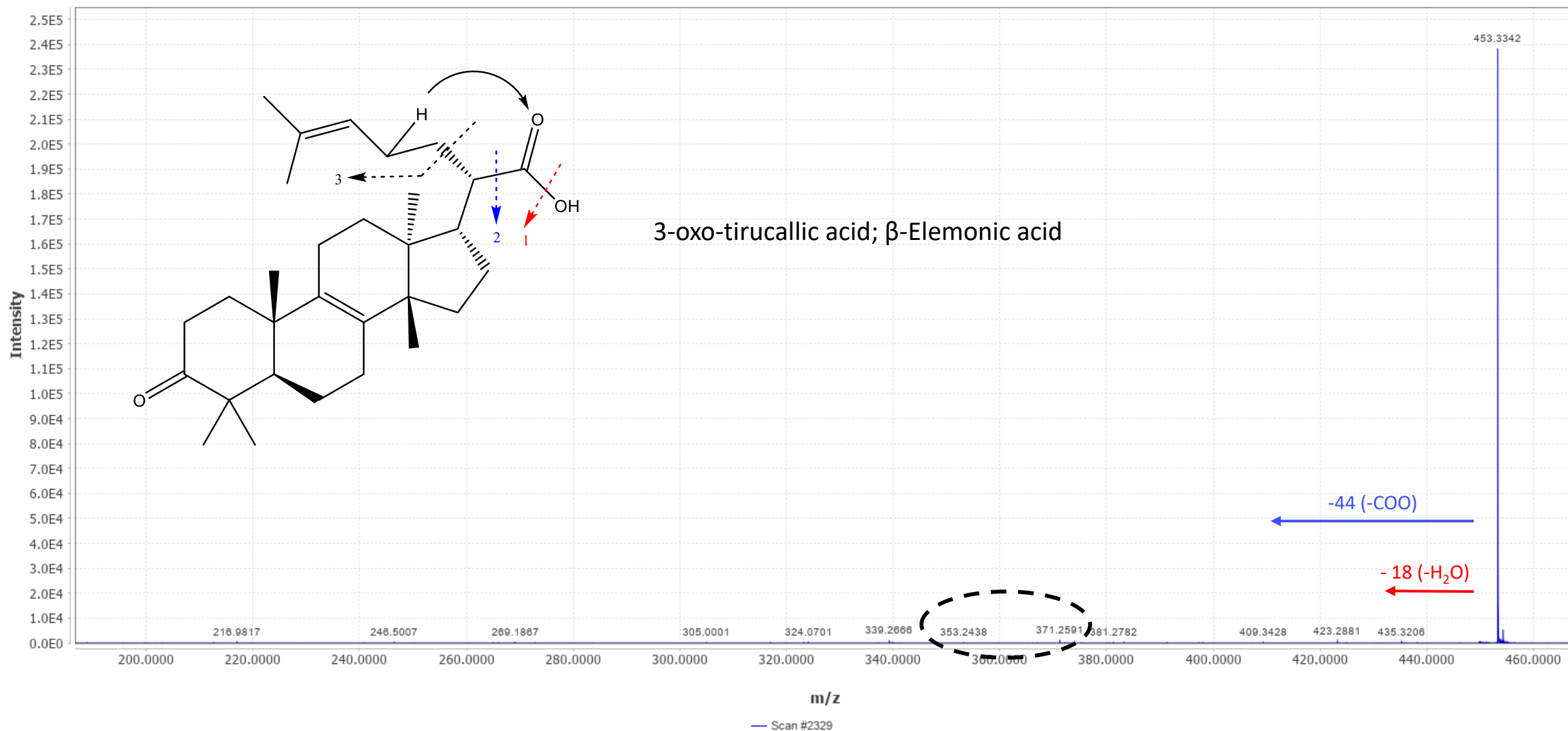
Neg_KM-Frank.mzML#1957 @5.36 MS2 (527.3363) p -, base peak: 527.3364 m/z (2.3E3)

Scan definition: scanId=321575



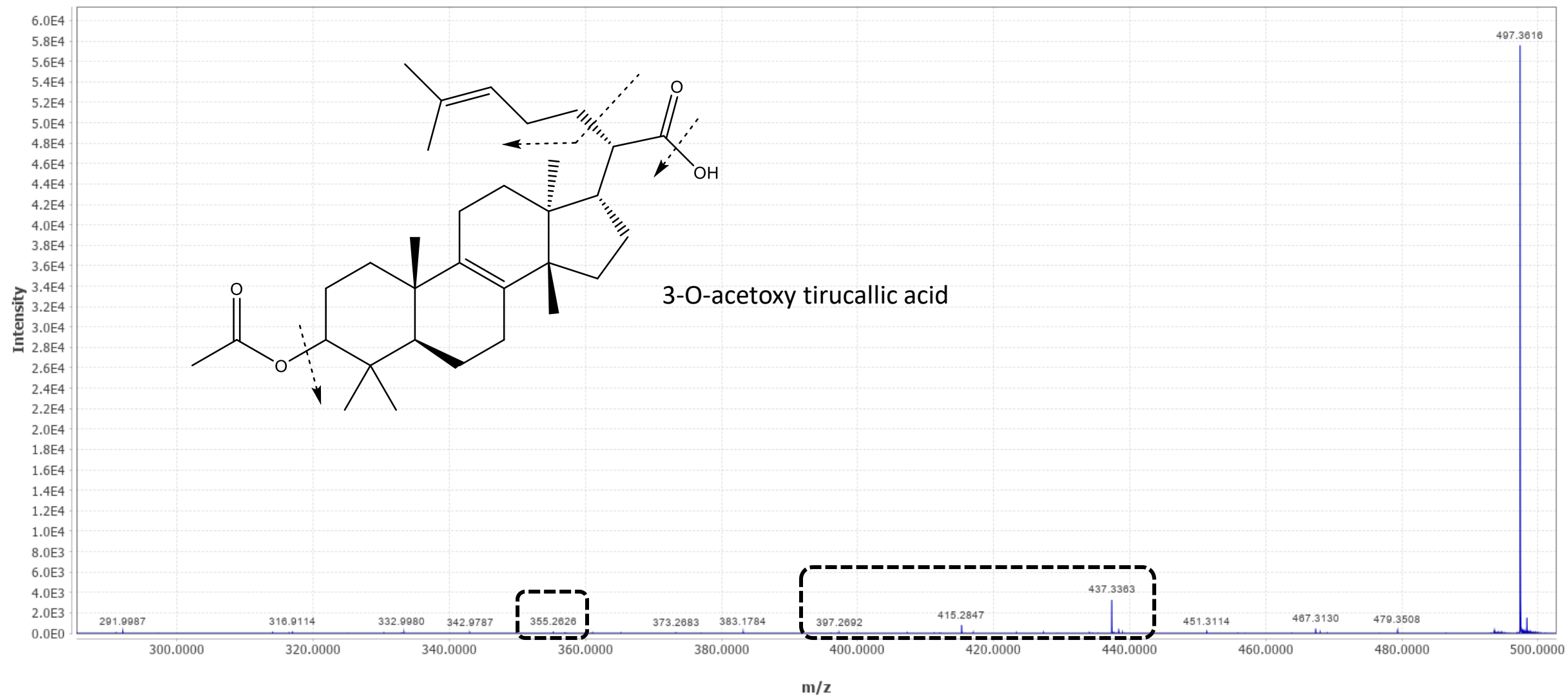
Neg_KM-Frank.mzML#2329 @6.39 MS2 (453.3363) p -, base peak: 453.3342 m/z (2.4E5)

Scan definition: scanId=383417



Neg_KM-Frank.mzML#2449 @6.72 MS2 (497.3623) p -, base peak: 497.3616 m/z (5.8E4)

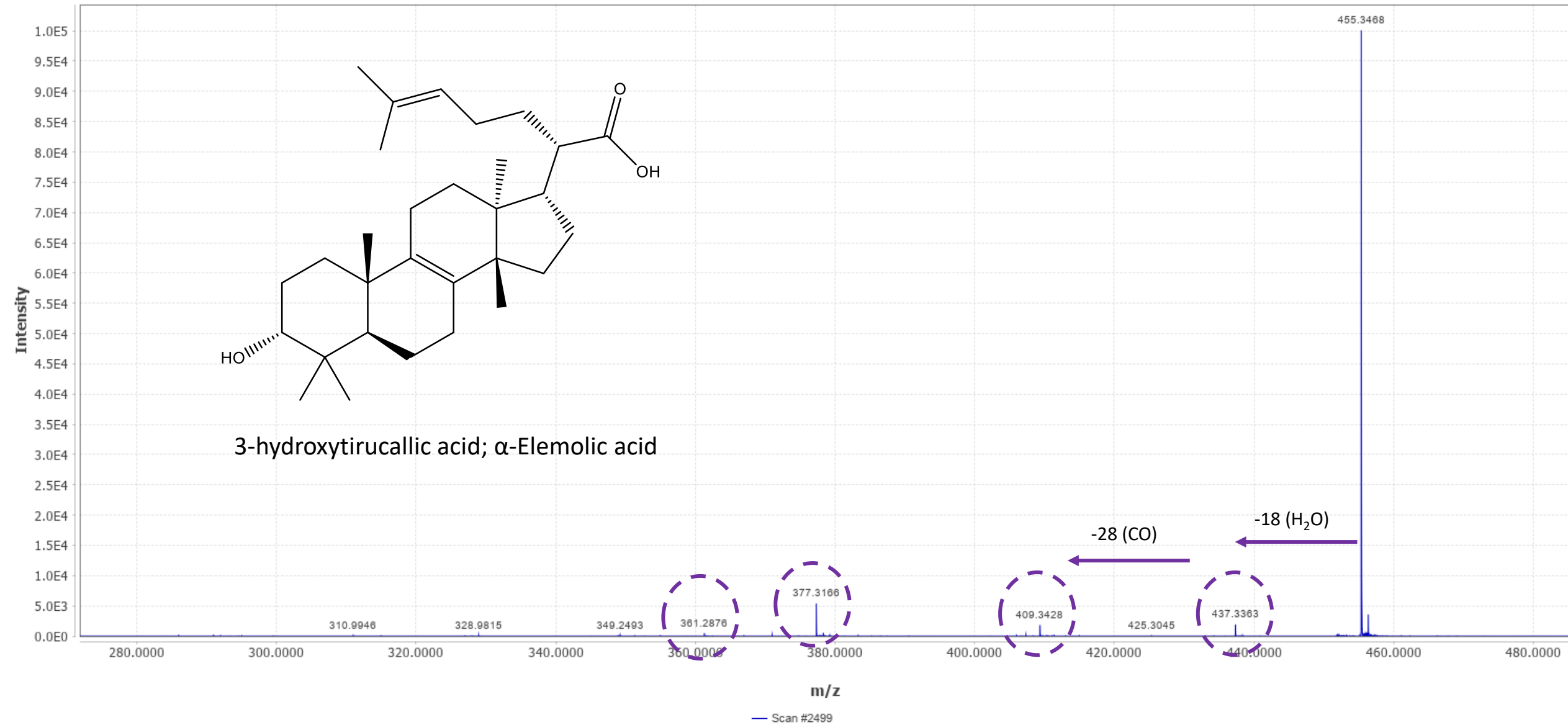
Scan definition: scanId=403366



— Scan #2449 ■ Peaks in Neg_KM-Frank.mzML chromatograms deconvoluted deisotoped

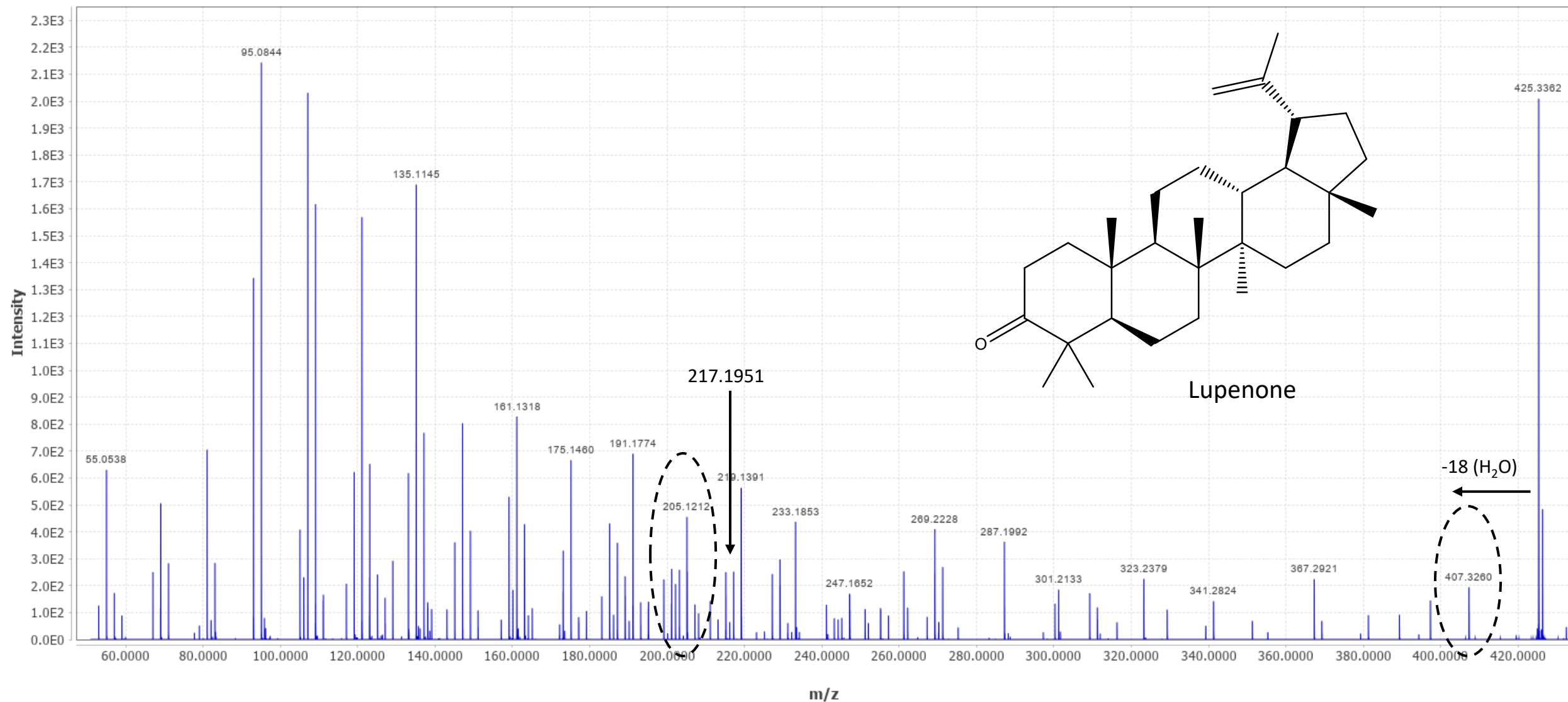
Neg_KM-Frank.mzML#2499 @6.86 MS2 (455.3518) p -, base peak: 455.3468 m/z (1.0E5)

Scan definition: scanId=411678



Pos_KM-Frank.mzML#2458 @6.77 MS2 (425.3772) p +, base peak: 95.0844 m/z (2.1E3)

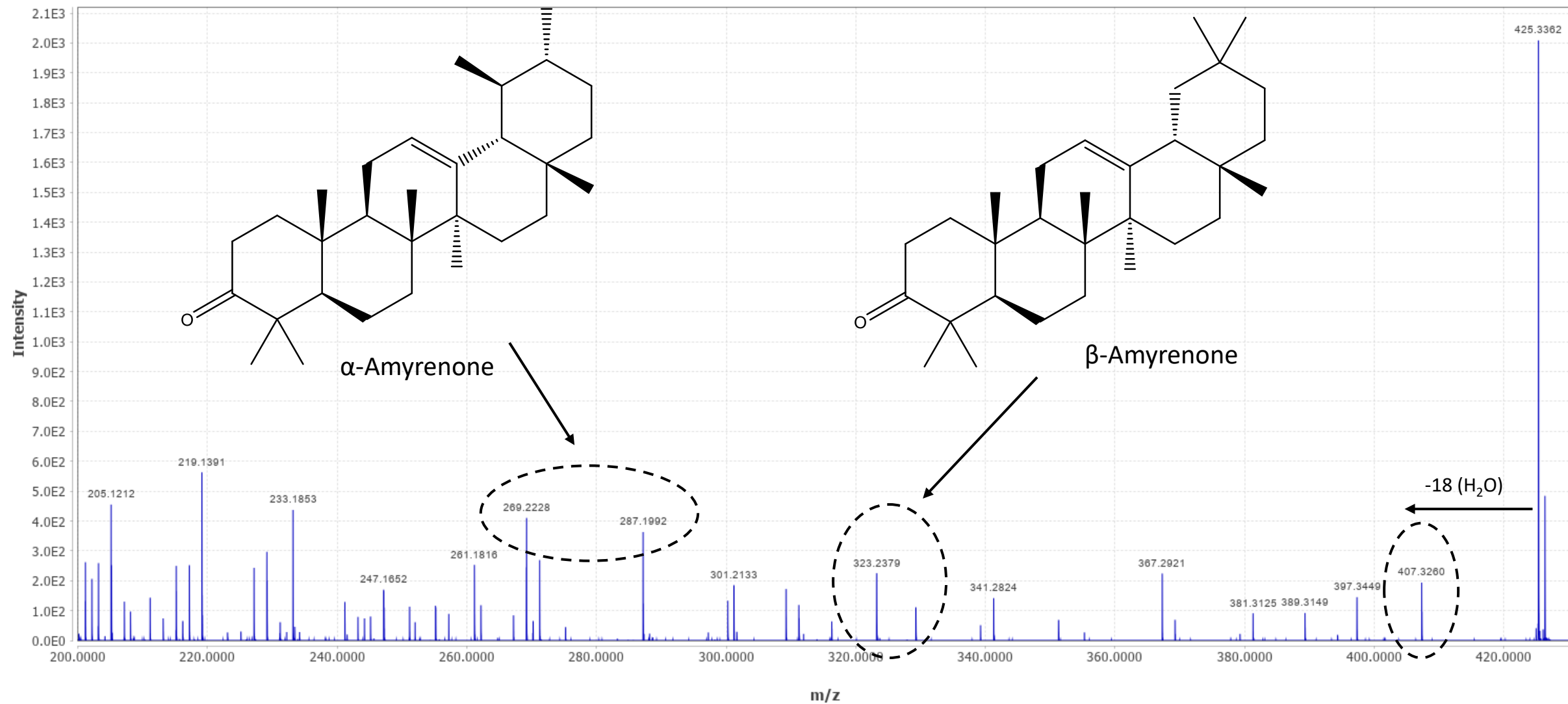
Scan definition: scanId=405958



— Scan #2458 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped

Pos_KM-Frank.mzML#2458 @6.77 MS2 (425.3772) p +, base peak: 95.0844 m/z (2.1E3)

Scan definition: scanId=405958



— Scan #2458 ■ Peaks in Pos_KM-Frank.mzML chromatograms deconvoluted deisotoped