Analysis of mononucleotides by tandem mass spectrometry: investigation of fragmentation pathways for phosphate- and ribose-modified nucleotide analogues

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Supplementary File

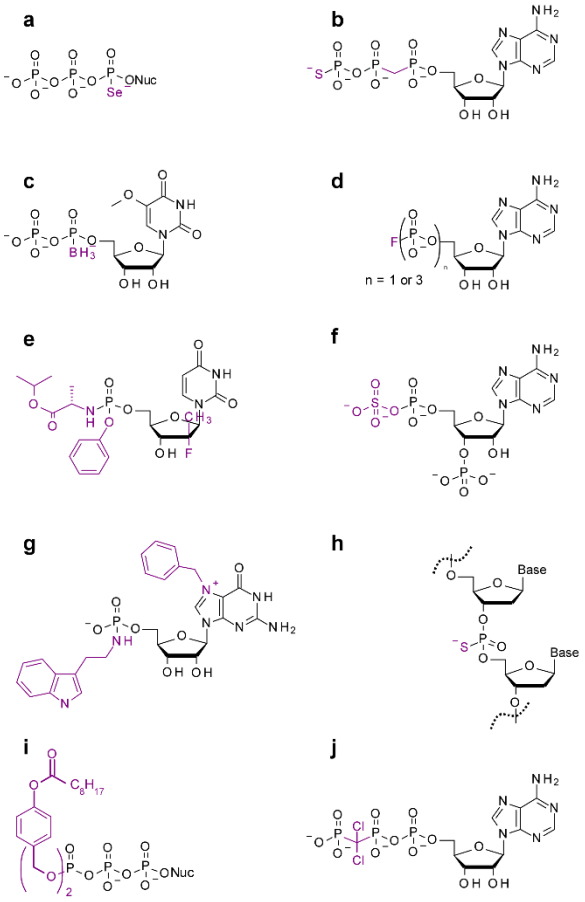


Fig. S1. **Examples of naturally occurring phosphate- and ribose-modified nucleotides or synthetic analogues with biological and therapeutic applications.** a) NTPαSe – polymerase substrates for nucleic acids modification[1]; b) ATPαS,β-γCH_2 – chelating agent with potential in Alzheimer's treatment[2]; c) 5-*O*-MeUDPαBH_3 – agonist of P2Y receptor[3]; d) NMPF, NDPF, and NTPF – unnatural substrates of HIT family enzymes[4]; e) sofosbuvir – anti-viral drug[5]; f) PAPS – natural compound, universal sulfotransferase cofactor[6]; g) 4E-i – prodrug form of translation inhibitor targeting eIF4E protein[7,8]; h) phosphorothioate DNA – synthetic modification used for therapeutic oligonucleotide (ON) stabilization, later discovered in bacteria[9]; i) ProPPNucleotides – cell-permeable NTP prodrugs[10]; j) ATPβ-γCCl_2 – metabolite of bisphosphonate anti-osteoporotic drug, clodronate[11-13]. Nuc = 5'-nucleosidyl moiety, Base = Adenine, Guanine, Cytosine, or Thymine.

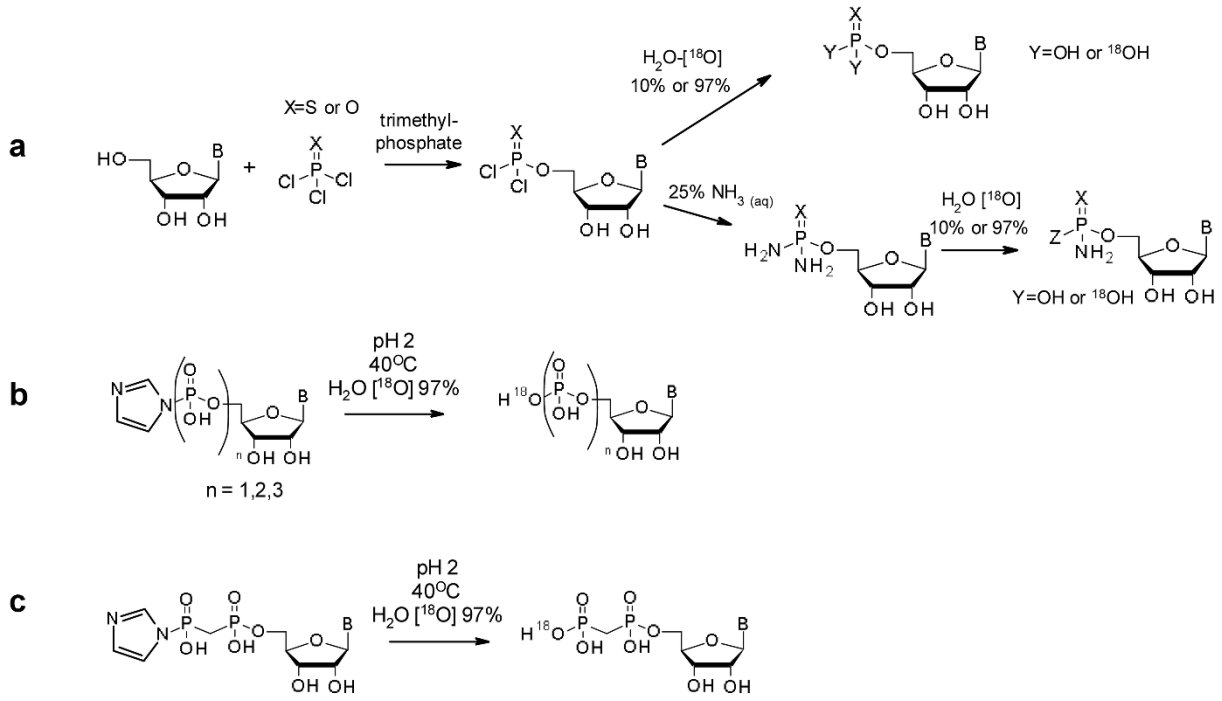


Fig. S2. **Syntheses of isotopically labelled nucleotides carried out in this work:** A - Nucleoside 5'-phosphorylation, nucleoside 5'-thiophosphorylation, nucleoside 5'-amidophosphorylation, B and C - P-Imidazolide hydrolysis.

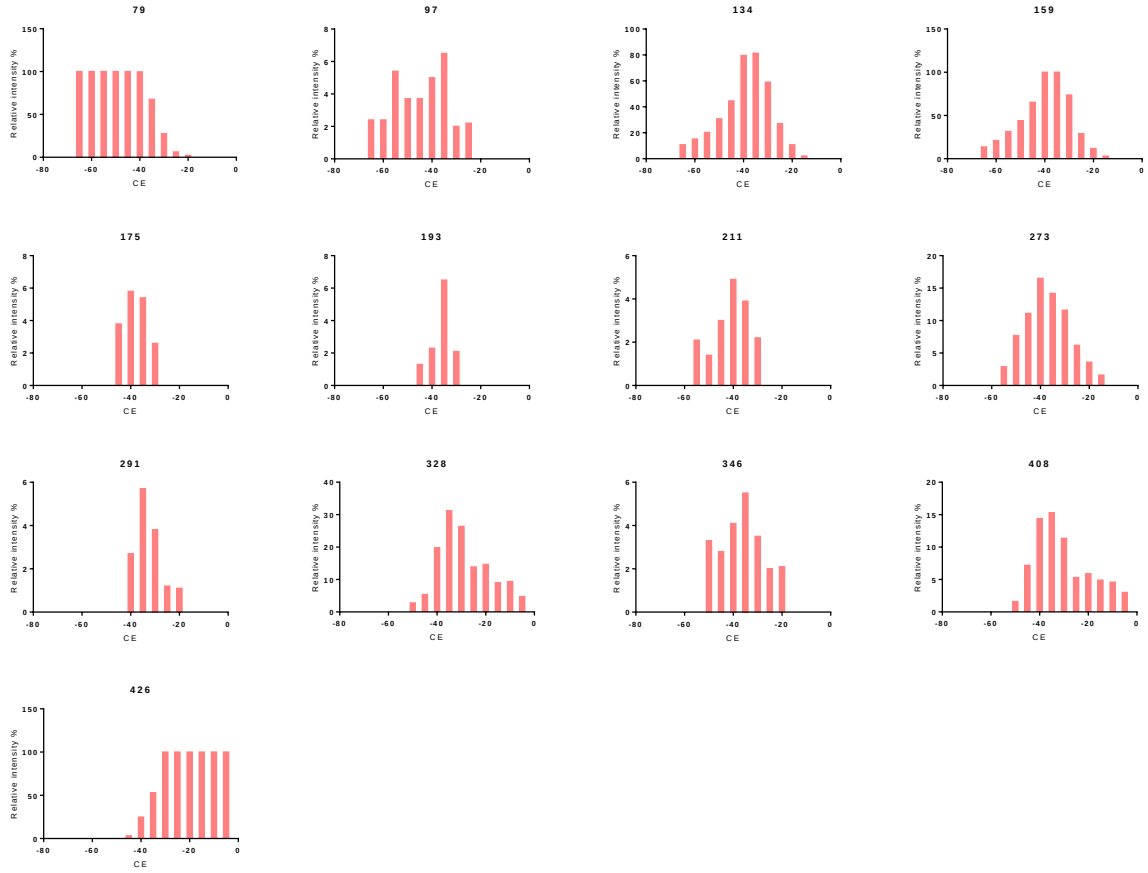


Fig. S3. **Fragment ion relative intensity distribution depending on Collision Energy (CE) for ADP.**

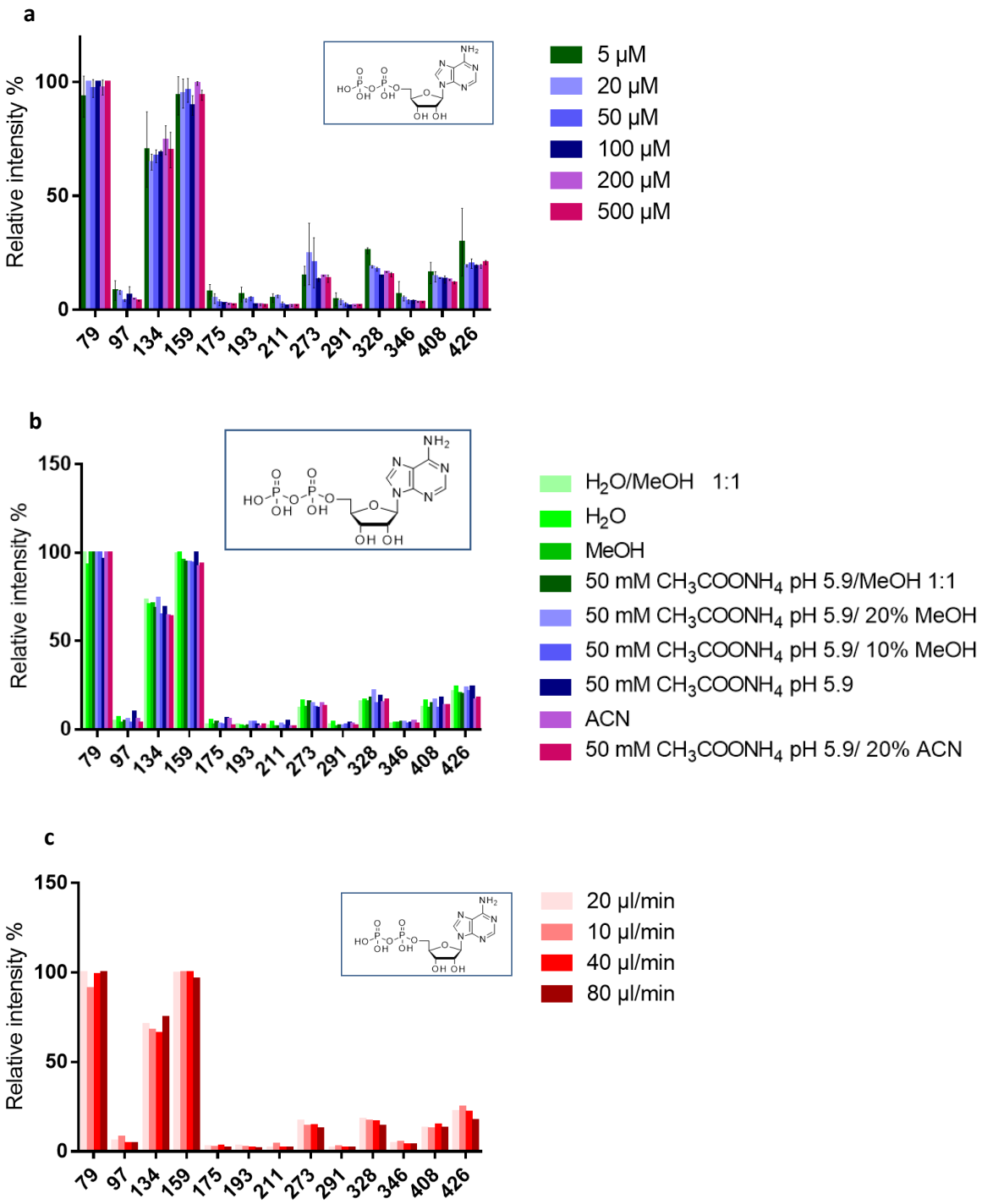


Fig. S4. **Relative ion intensities in ESI(-) MS/MS spectra** of: A) ADP dissolved in 50 mM CH_3COONH_4 pH 5.9/ 20% MeOH at various concentrations. The data shown are mean relative intensities from duplicate experiments +/- S.D. B) 50 µM ADP depending on solvent composition. C. 50 µM ADP in 50 mM CH_3COONH_4 pH 5.9/ 20% MeOH depending on infusion flow rate.

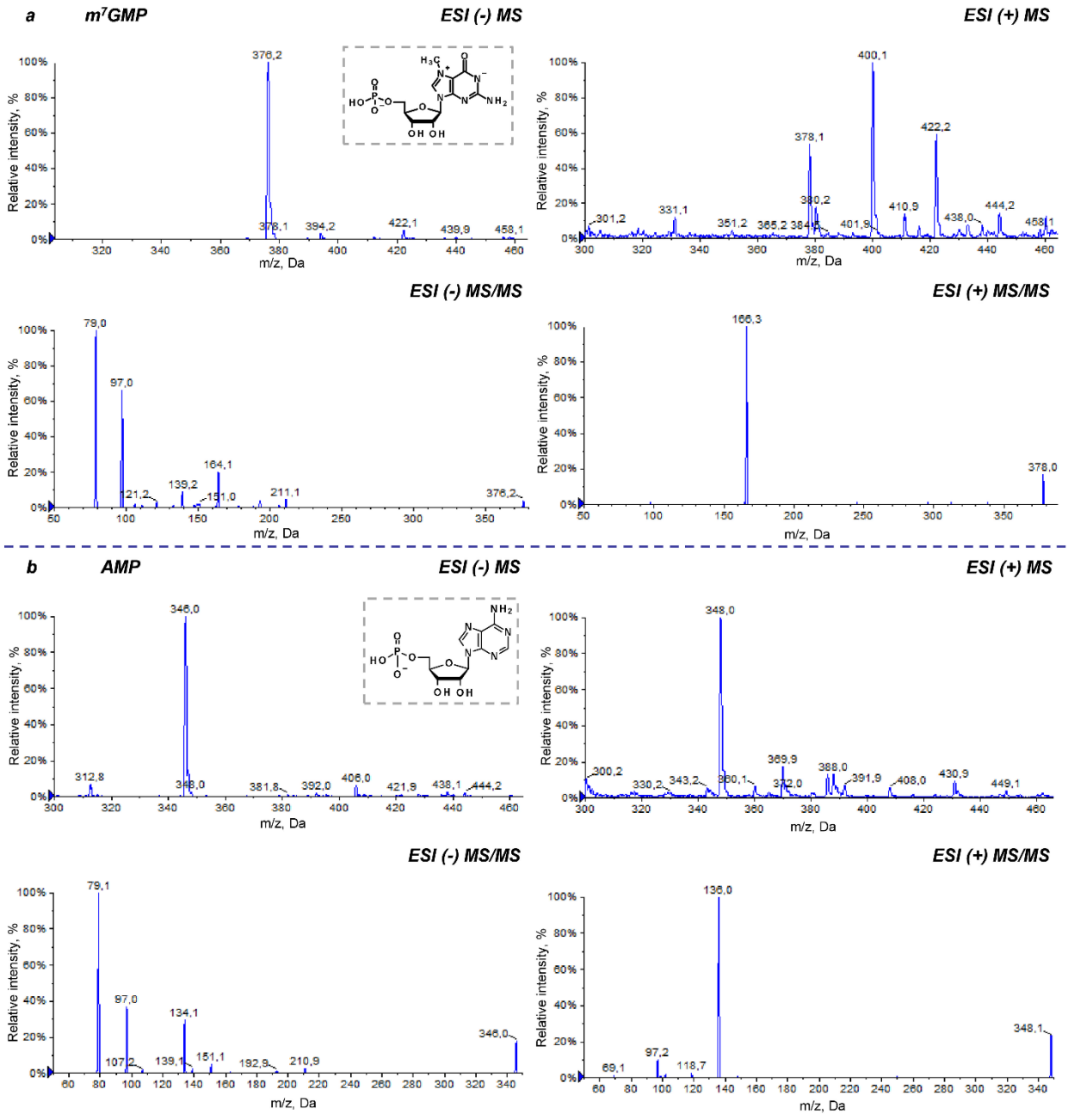


Fig. S5. **Comparison of MS and MS/MS spectra in positive and negative ion mode for m^{7}GMP (A) and AMP (B).**

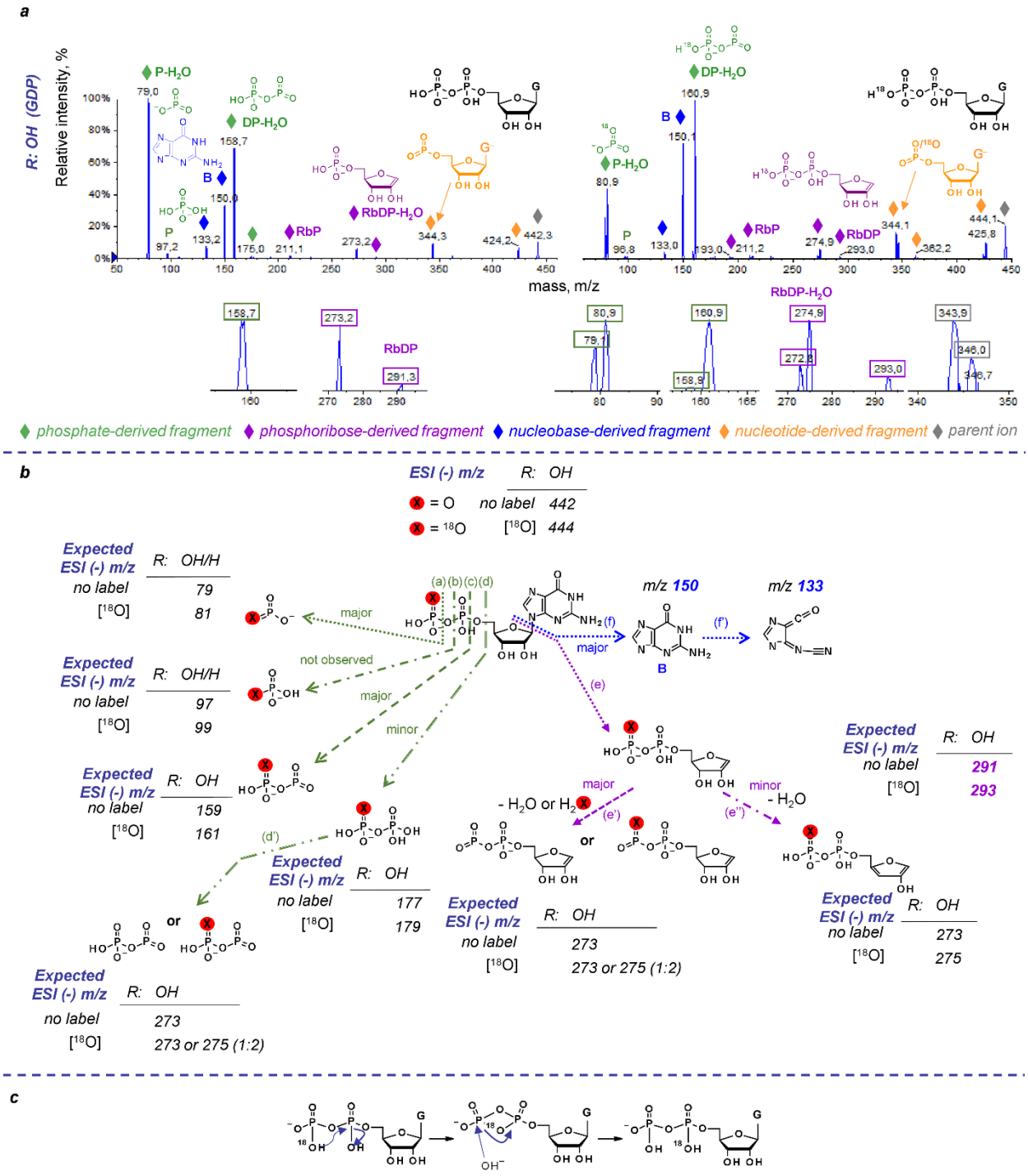


Fig. S6. **Negative-ion mode fragmentation of nucleoside 5'-diphosphates**. a) ESI(-)/MS/MS spectra of GDP and its β-[^{18}O]-labelled analogue. b) Proposed fragmentation pathways for NDPs, exemplified by the fragmentation of GDP. c) Possible mechanism for the transfer of [^{18}O] from the β-phosphate to α-phosphate, explaining the presence of [^{18}O]-labelled guanosine monophosphate derivatives in the MS/MS spectrum of β-[^{18}O]-GDP.

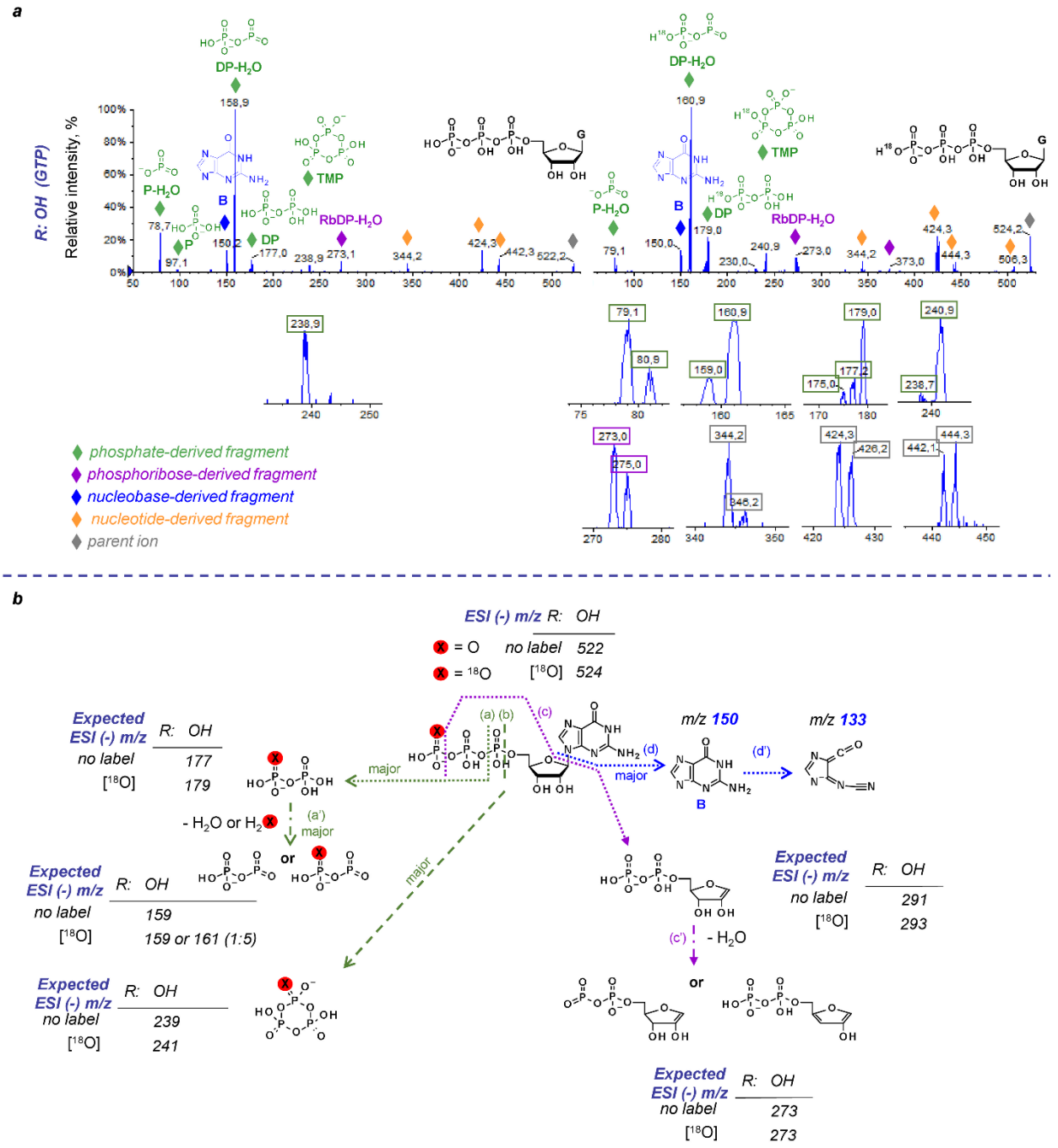


Fig. S7. **Negative-ion mode fragmentation of nucleoside 5'-triphosphates.** a) ESI(-)/MS/MS spectra of GTP and its γ-[^{18}O]-labelled analogue. b) Proposed fragmentation pathways for NTPs, exemplified by the fragmentation of GTP.

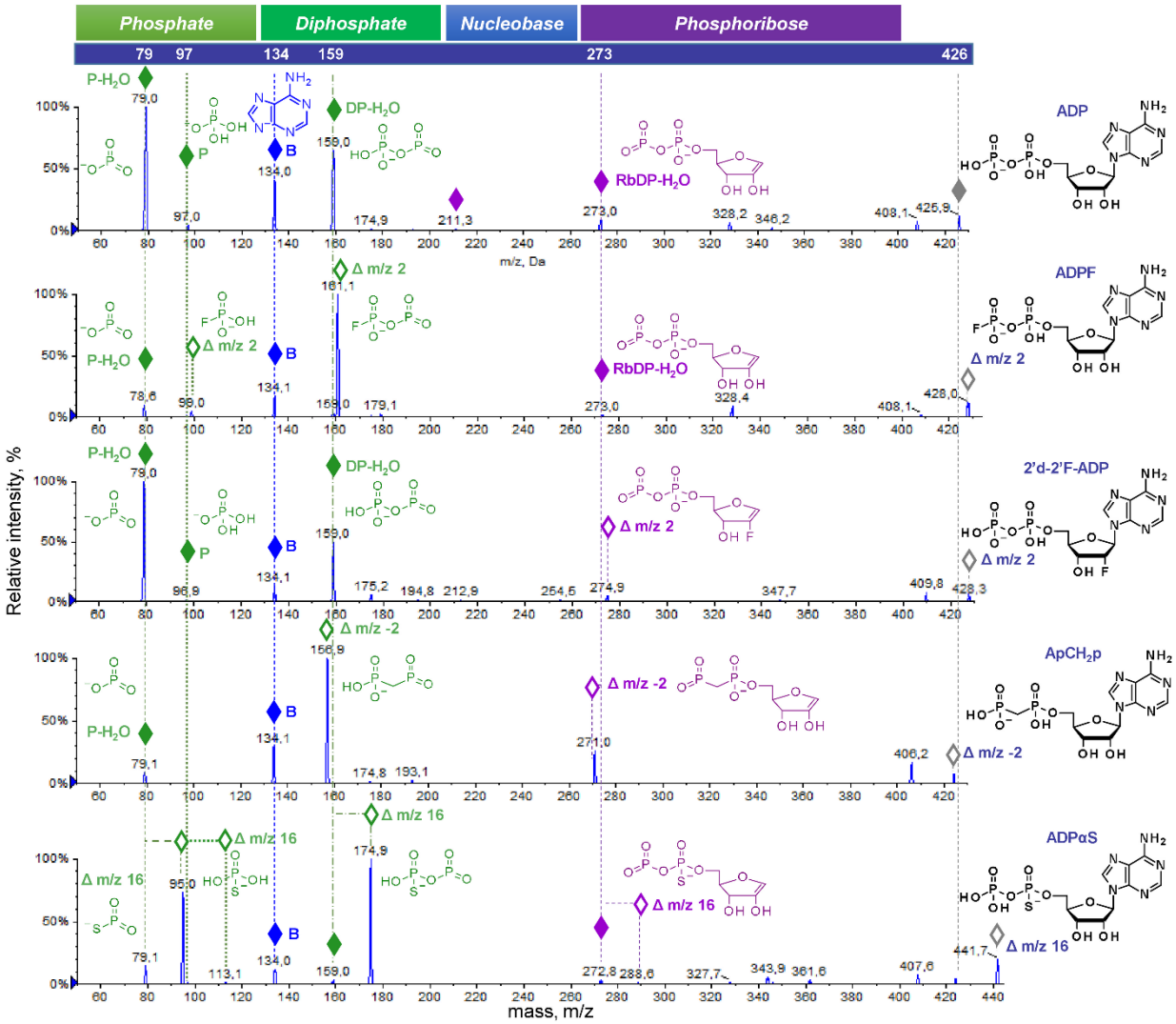


Fig. S8. **Analysis of ADP and its phosphate- and ribose-modified analogues by ESI(-)/MS/MS.** In each spectrum, fragmentation ions enabling determination of the substitution site (nucleobase *versus* phosphate *versus* ribose) are indicated with diamonds (◊) and their proposed structures are depicted.

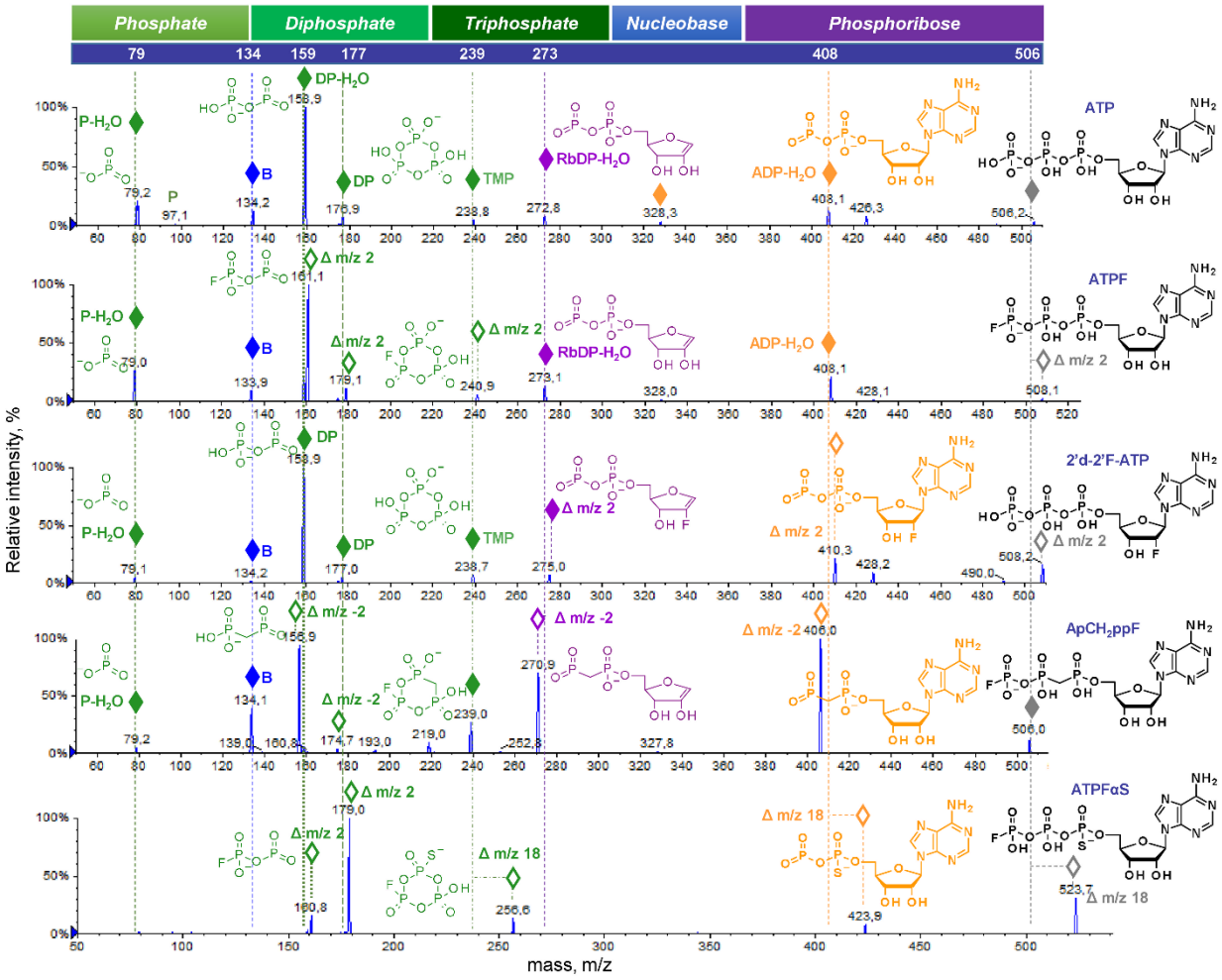


Fig. S9. **Analysis of ATP and its phosphate- and ribose-modified analogues by ESI(-)/MS/MS.** In each spectrum, fragmentation ions enabling determination of the substitution site (nucleobase *versus* phosphate *versus* ribose) are indicated with diamonds (◊) and their proposed structures are depicted.

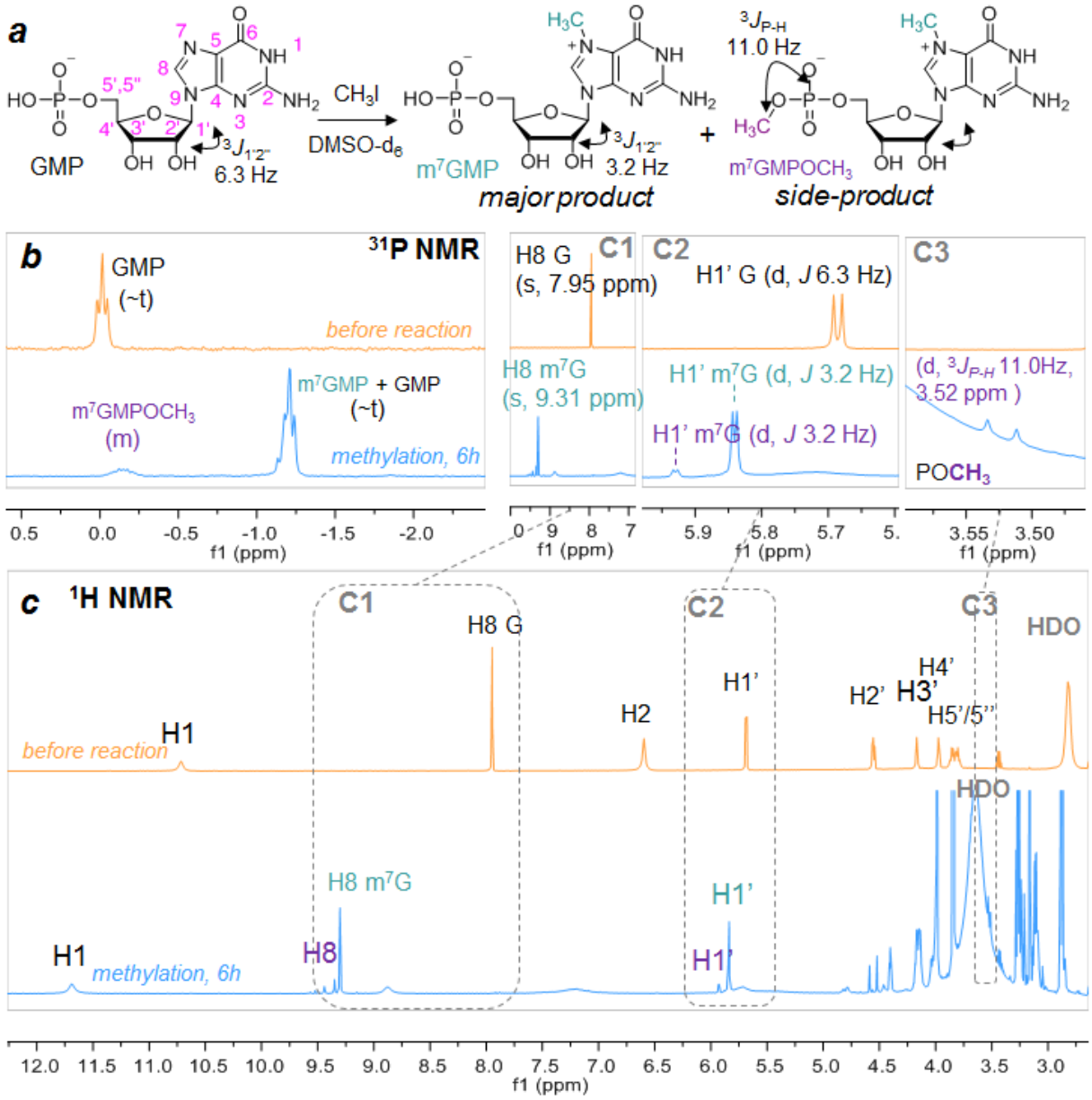


Fig. S10. **Analysis of GMP methylation reaction by ^{31}P and ^{1}H NMR.** A) Reaction scheme proposed based on HPLC and ESI (-) MS/MS analysis (Fig. 7, main manuscript); B) Analysis of reaction progress by ^{31}P NMR – indicated are signals crucial for structure confirmation; C) Analysis of reaction progress by ^{1}H NMR – indicated are signals crucial for structure confirmation.

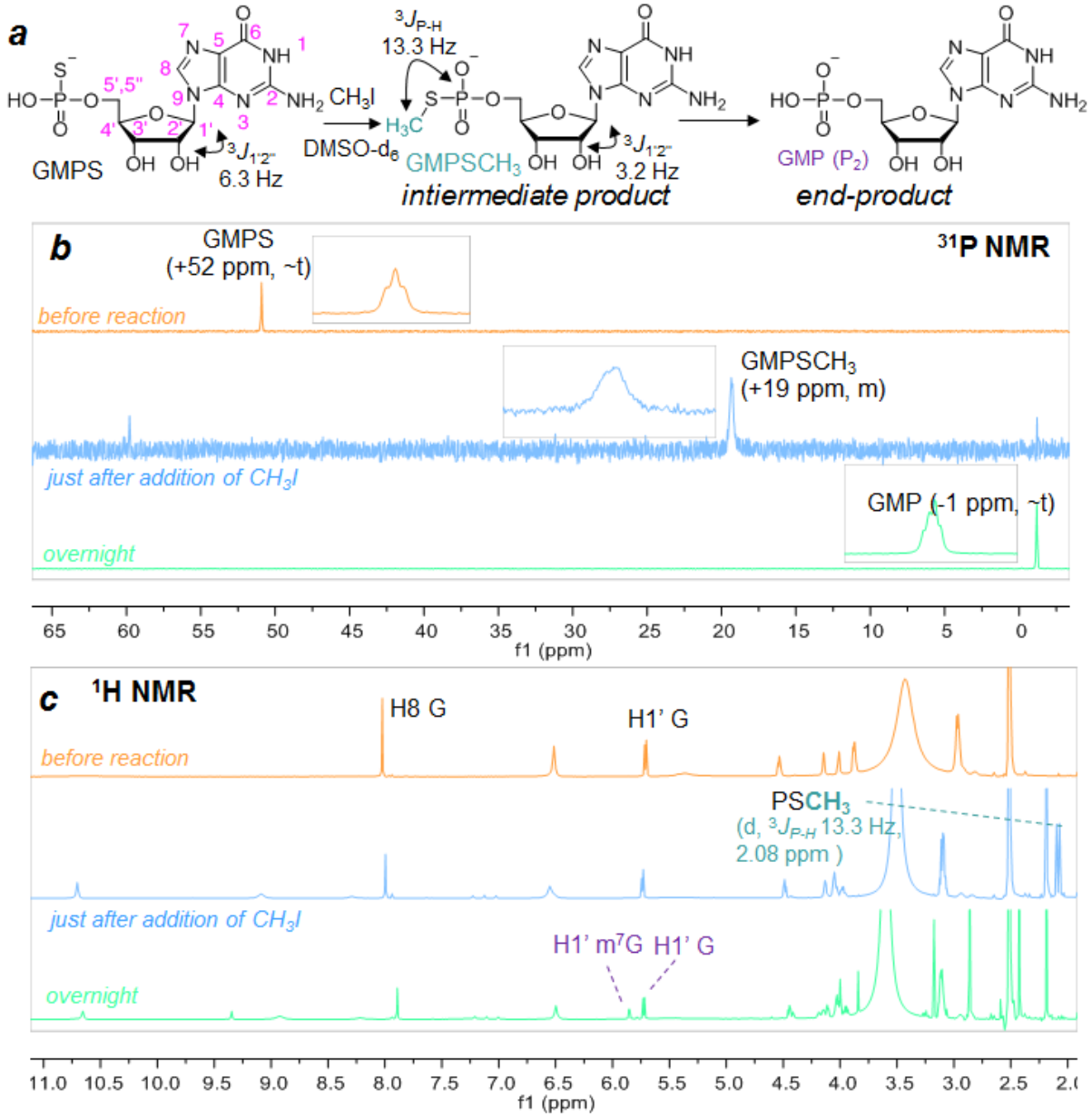
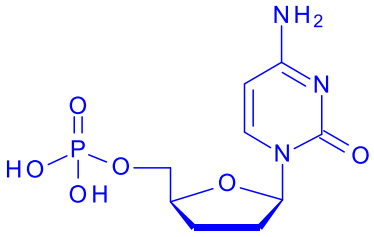
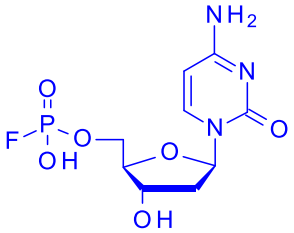
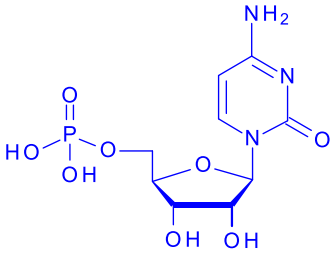
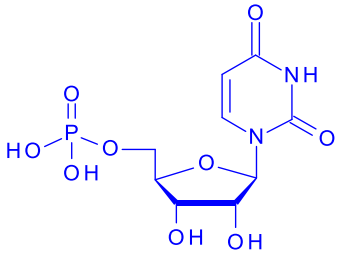
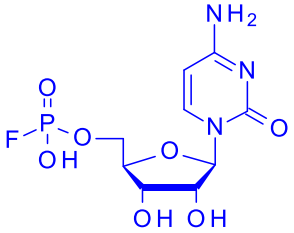
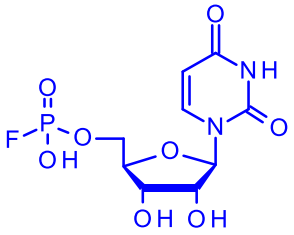
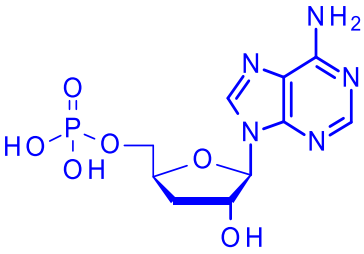
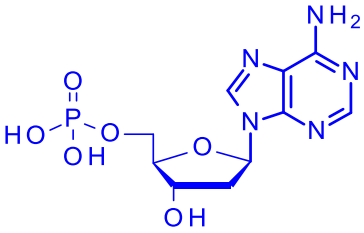
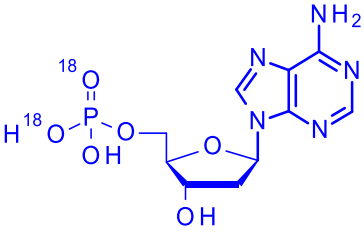
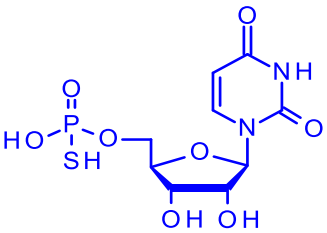
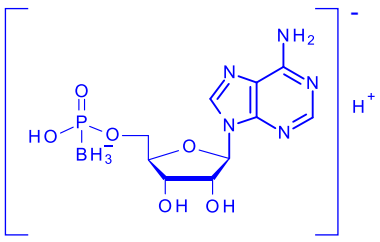
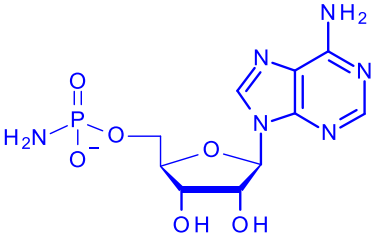
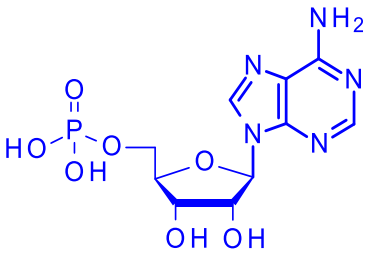
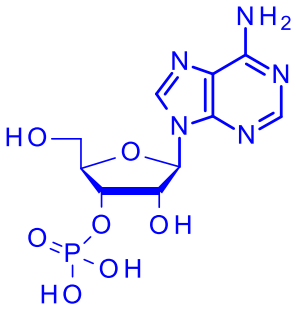
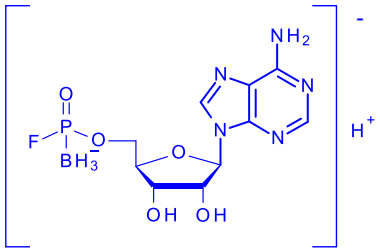
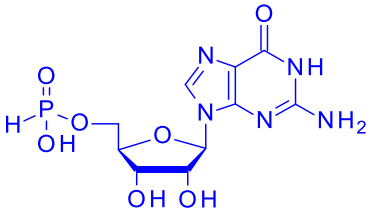
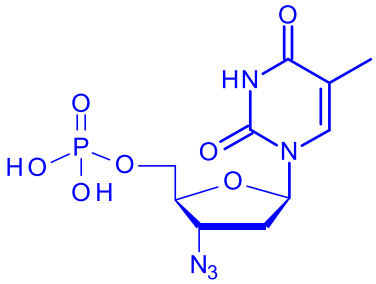
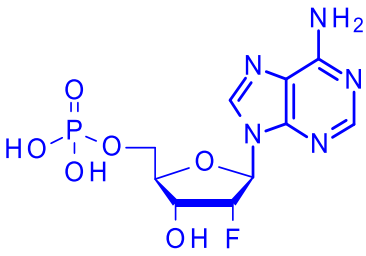


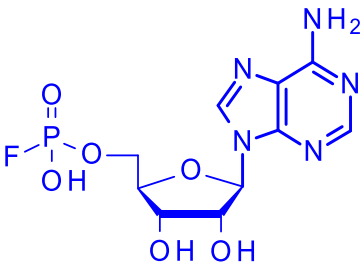
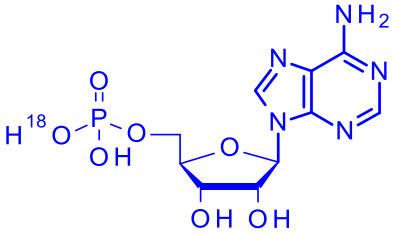
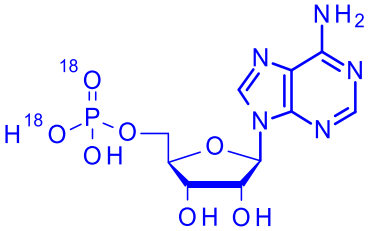
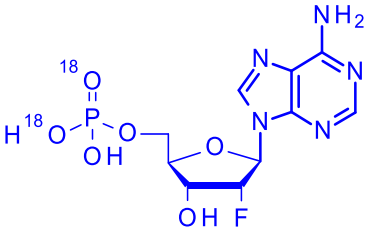
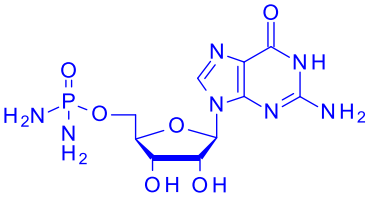
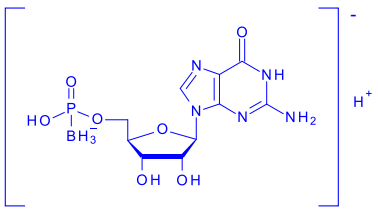
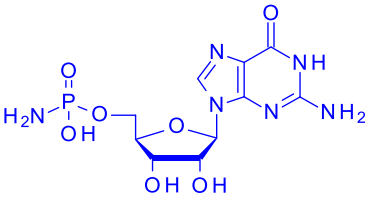
Fig. S11. **Analysis of GMPS methylation reaction by ^{31}P and ^{1}H NMR.** A) reaction scheme proposed based on HPLC and ESI (-) MS/MS analysis (Fig. 7, main manuscript); B) Analysis of reaction progress by ^{31}P NMR – indicated are signals crucial for structure confirmation; C) Analysis of reaction progress by ^{1}H NMR – indicated are signals crucial for structure confirmation.

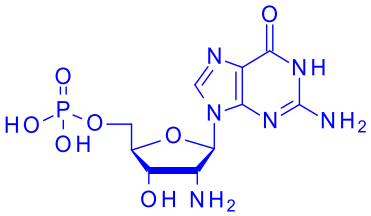
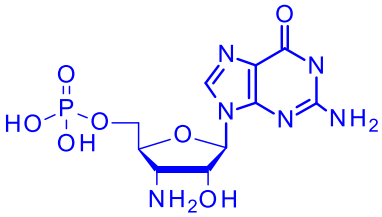
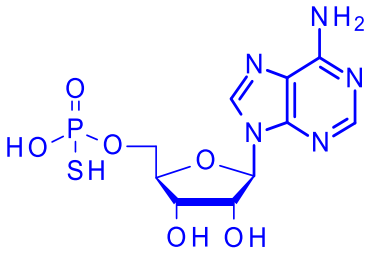
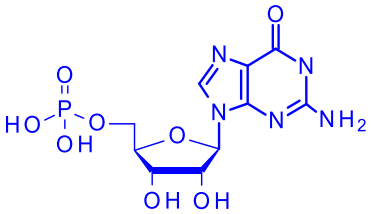
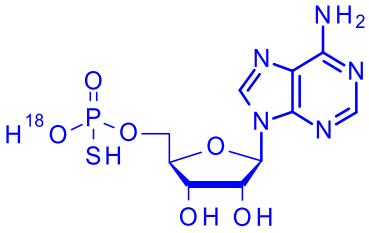
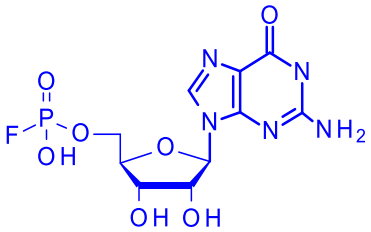
Table S1. **Names and structures of investigated compounds.**

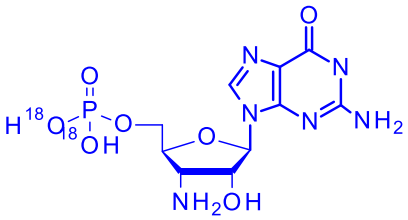
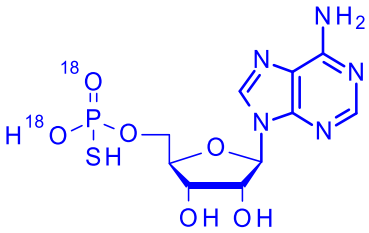
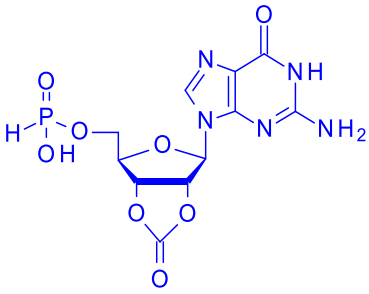
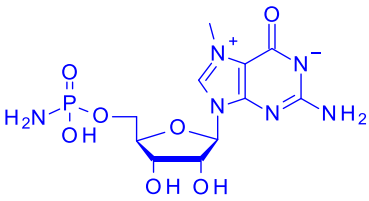
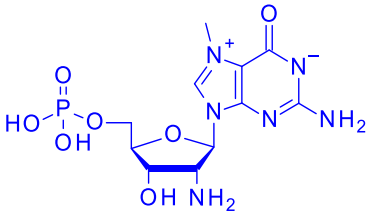
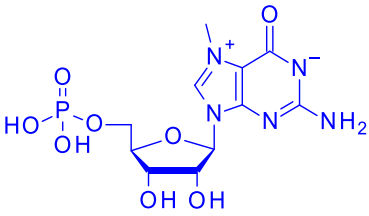
Nr	Abbreviation	Name	Structure	M	Flow rate µl/min	Concentration µM	Ref.[a]
1.	ddCMP	2′,3′-dideoxycytidine 5'-monophosphate		291	10	100	(YOSHIKAW. M et al., 1967)
2.	2'-dCMPF	2'-deoxycytidine 5′-fluoromonophosphate		309	10	100	(Baranowski et al., 2015)
3.	CMP	Cytidine 5'-monophosphate		323	10	100	(YOSHIKAW. M et al., 1967)
4.	UMP	Uridine 5'-monophosphate		324	10	100	(YOSHIKAW. M et al., 1967)
5.	CMPF	Cytidine 5′-fluoromonophosphate		325	20	150	(Baranowski et al., 2015)
6.	UMPF	Uridine 5′-fluoromonophosphate		326	20	100	(Baranowski et al., 2015)

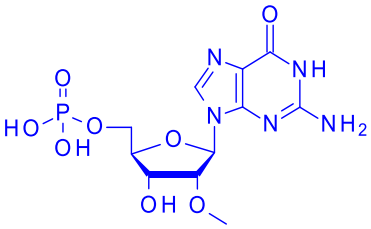
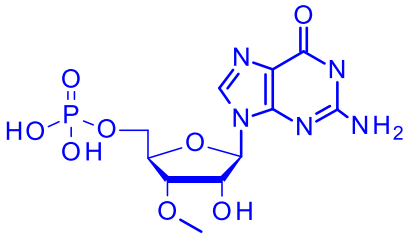
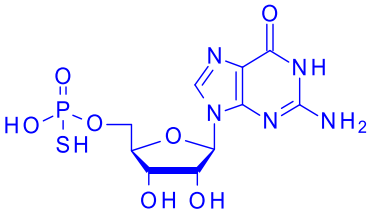
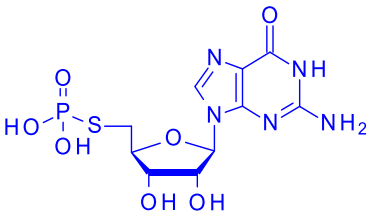
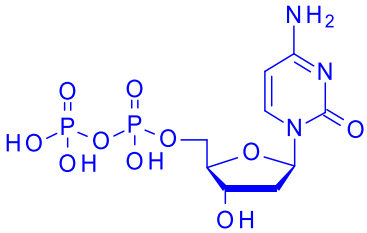
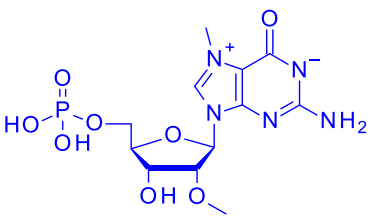
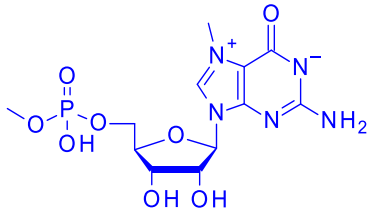
7.	3'-dAMP	3’-deoxyadenosine 5’-monophosphate		331	20	30	Commercial (Sigma Aldrich)
8.	2’-dAMP	2’-deoxyadenosine 5’-monophosphate		331	50	100	Commercial (Sigma Aldrich)
9.	[^{18}O,^{18}O]2’-dAMP	2’deoxyadenosine 5’-([^{18}O, ^{18}O]-monophosphate)		335	100	100	Experimental
10.	UMPS	Uridine 5′-thiomonophosphate		340	100	100	(Kowalska et al., 2008)
11.	AMPαBH$_3$	Adenosine 5′-boranomonophosphate		345	10	100	(Kowalska et al., 2014)
12.	AMPNH$_2$	Adenosine 5′-amidophosphate		346	10	150	Experimental

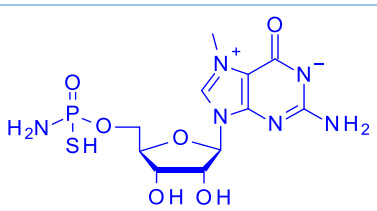
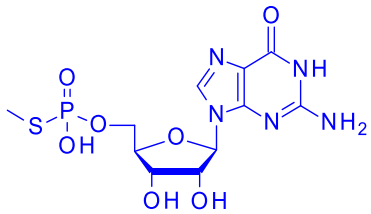
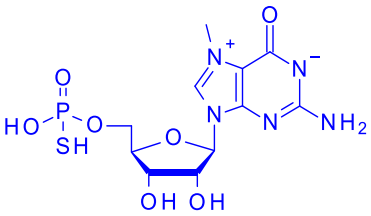
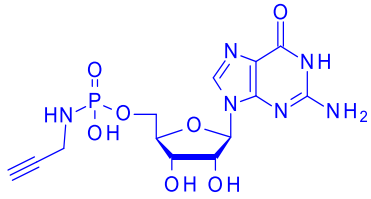
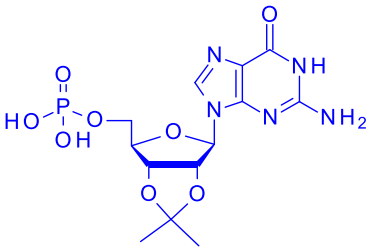
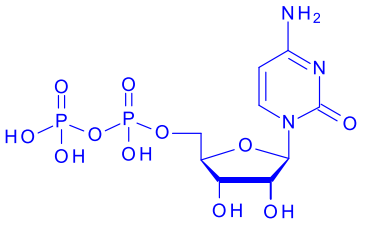
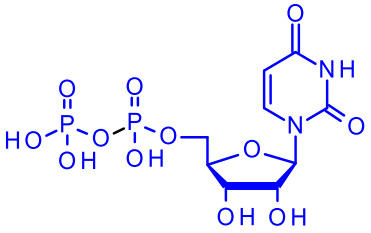
13.	5’-AMP, AMP	Adenosine 5’-monophosphate		347	20	50	Commercial (Sigma-Aldrich)
14.	3'-AMP	Adenosine 3’-monophosphate		347	100	100	Commercial (Sigma Aldrich)
15.	AMPF αBH_3	Adenosine 5′-fluoroboranomonophosphate		347	20	50	(Baranowski et al., 2015)
16.	GMPH	Guanosine 5’-H-phosphonate		347	20	50	(Strenkowska et al., 2012)
17.	AZTMP	3′-azido-3′-deoxythymidine 5'-monophosphate		347	10	50	(YOSHIKAW. M et al., 1967)
18.	2'-F, 2’-dAMP	2’-fluoro-2’-deoxyadenosine 5’-monophosphate		349	50	100	Experimental

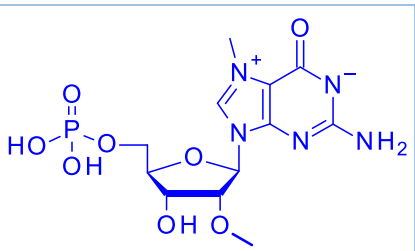
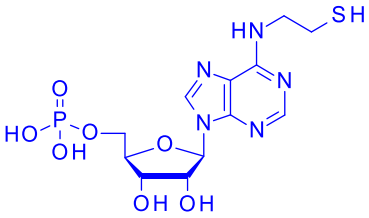
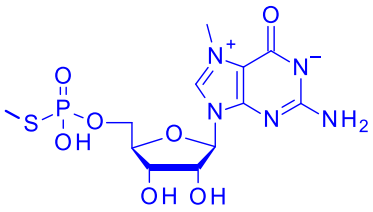
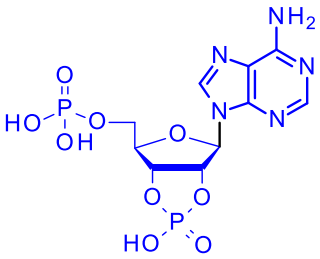
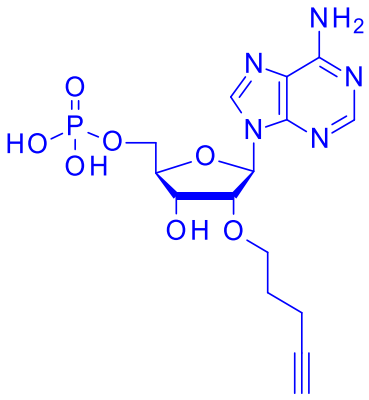
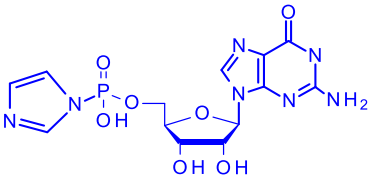
19.	AMPF	Adenosine 5′-fluoromonophosphate		349	100	50	(Baranowski et al., 2015)
20.	[^{18}O] AMP	Adenosine 5’-([^{18}O]-monophosphate)		349	100	100	Experimental
21.	[^{18}O,^{18}O]AMP	Adenosine 5’- ([^{18}O, ^{18}O]-monophosphate)		351	100	100	Experimental
22.	[^{18}O,^{18}O]2'-F, 2’-dAMP	2’-fluoro-2’-deoxyadenosine ([^{18}O, ^{18}O]-monophosphate)		353	50	100	Experimental
23.	GMP(NH_2)$_2$	Guanosine 5′-phosphorodiamidate		361	10	50	Experimental
24.	$GMPBH_3$	Guanosine 5′-boranophosphate		361	10	100	(Kowalska et al., 2014)
25.	$GMPNH_2$	Guanosine 5′-amidophosphate		362	100	50	Experimental

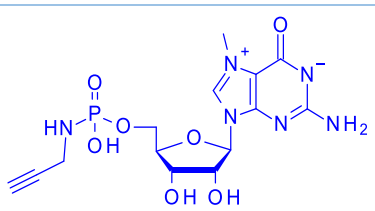
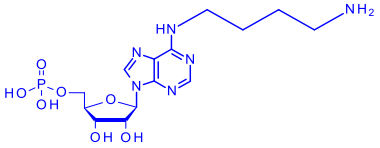
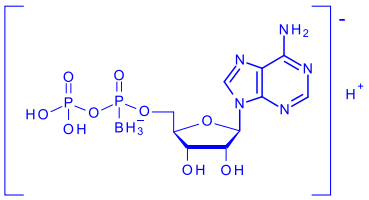
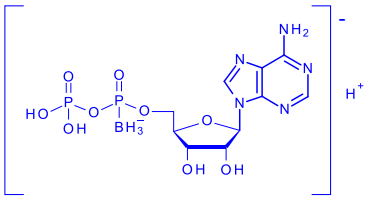
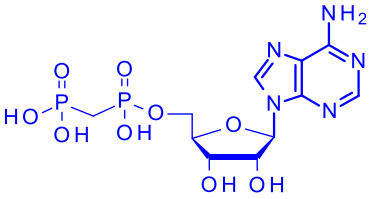
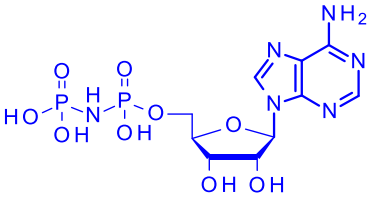
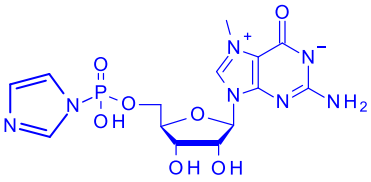
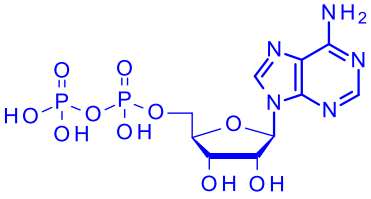
26.	2'-NH_2, 2'-dGMP	2'-amino-2'-deoxyguanosine 5'-monophosphate		362	70	30	(Jemielity et al., 2012)
27.	3'NH2GMP	3'-amino-3'-deoxyguanosine 5'-monophosphate		362	50	50	Experimental
28.	AMPS	Adenosine 5′-thiomonophosphate		363	20	50	(Kowalska et al., 2008)
29.	GMP	Guanosine 5'-monophosphate		363	100	100	Commercial (Sigma-Aldrich)
30.	[^{18}O]AMPS	adenosine 5′-([^{18}O]thiomonophosphate)		365	100	50	Experimental
31.	GMPF	Guanosine 5′-fluoromonophosphate		365	10	100	(Baranowski et al., 2015)

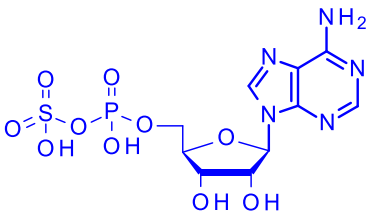
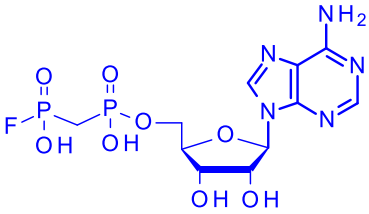
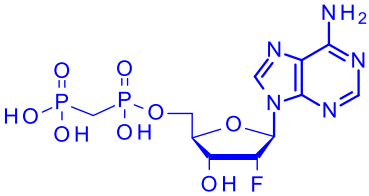
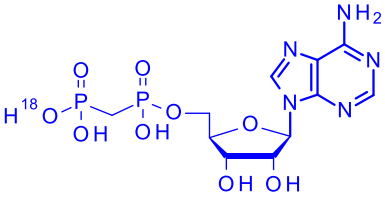
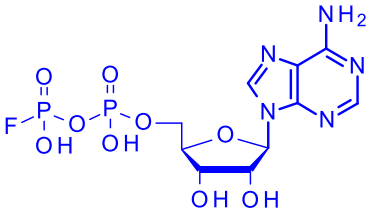
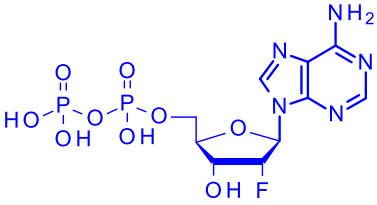
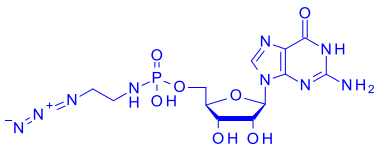
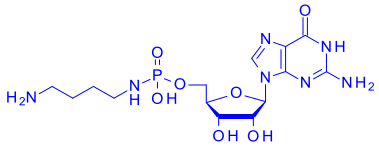
32.	[^{18}O,^{18}O]3'NH2 GMP	3'-amino-3'-deoxyguanosine 5'-([^{18}O,^{18}O]-monophosphate)		366	70	30	Experimental
33.	[[^{18}O,^{18}O] AMPS	Adenosine 5′-tiomonophosphate ^{18}O isotope		367	100	100	Experimental
34.	CO-GMPH	2',3'-O,O-carbonyl-guanosine 5'-H-phosphonate		373	20	50	Experimental
35.	m^{7}GMPNH$_2$	*N*7-methylguanosine 5′-amidophosphate		376	30	50	Experimental
36.	2'-NH$_2$, 2'-d m^{7}GMP	2'-amino-2'-deoxy-7-methylguanosine 5'-monophosphate		376	50	50	(Jemielity et al., 2012)
37.	m^{7}GMP	N7-methylguanosine 5′-monophosphate		377	10	30	(Jemielity et al., 2003)

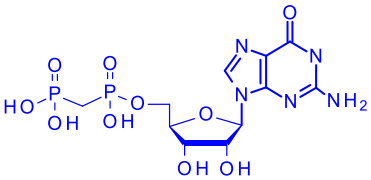
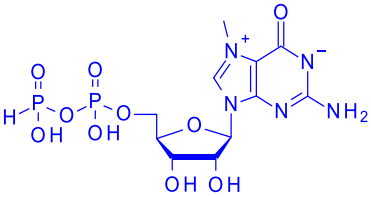
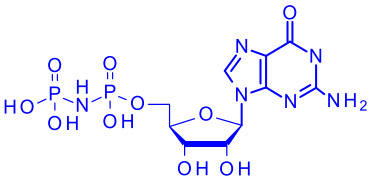
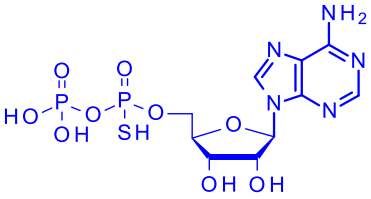
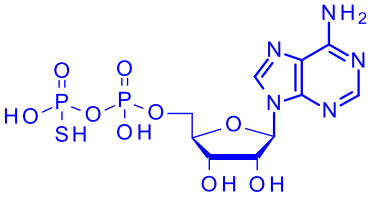
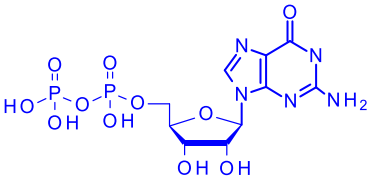
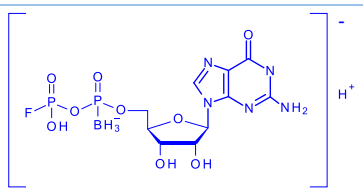
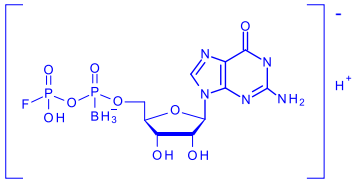
38.	$m^{2'\text{-}O}GMP$	2'-*O*-methylguanosine 5'-monophosphate		377	100	50	(Jemielity et al., 2003)
39.	3'-OMeGMP	3'-O-methylguanosine 5'-monophosphate		377	100	50	(Stepinski et al., 2001)
40.	GMPS	Guanosine 5′-thiomonophosphate		379	20	100	(Kowalska et al., 2008)
41.	GMSP	Guanosine 5'-phosphorothiolate		379	10	100	Wojtczak et. al. in preparation
42.	2'-dCDP	2'deoxycytidine 5′-diphosphate		387	10	100	Commercial (Sigma Aldrich)
43.	$m_2{}^{7,2'\text{-}O}GMP$	*N*7,2'-O-dimethylguanosine		391	10	50	(Jemielity et al., 2003)
44.	$m^7GMPOCH_3$	*N*7-methylguanosine 5′-(*O*-methyl)monophosphate		391	100	100	(Jemielity et al., 2003)

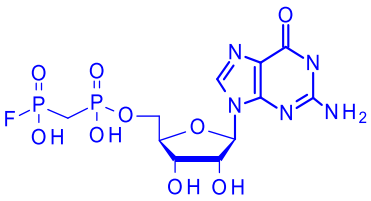
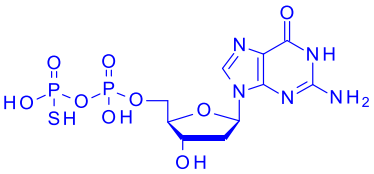
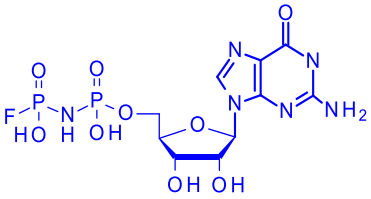
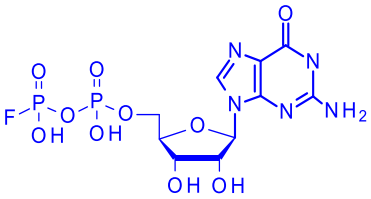
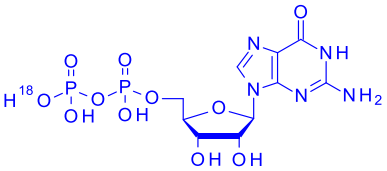
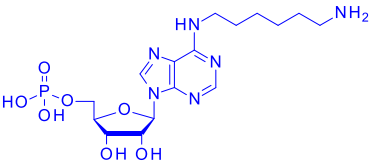
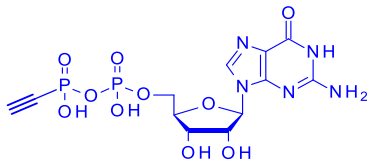
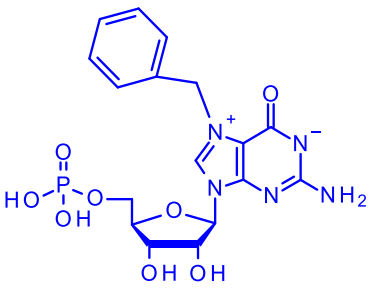
45.	$m^7GMPSNH_2$ D1	N7-methylguanosine 5'-phosphorothioamidate		392	10	30	Kopcial et al. in preparation
46.	$GMPSCH_3$	Guanosine 5′-O-(S-methyl)thiomonophosphate		393	10	100	(Kowalska et al., 2009)
47.	m^7GMPS	*N*7-methylguanosine 5′-thiomonophosphate		393	10	50	(Kowalska et al., 2008)
48.	$GMPNH_2CH_2CCH$	N-propargyl guanosine 5'-phosphoroamidate		400	10	100	(Walczak et al., 2017)
49.	iPr-GMP	2',3'-*O,O*-isopropylideneguanosine 5'-monophosphate		403	30	100	(Warminski et al., 2013)
50.	CDP	Cytidine 5′-diphosphate		403	20	50	Commercial (Sigma Aldrich)
51.	UDP	Uridine 5′-diphosphate		404	10	100	Commercial (Sigma Aldrich)

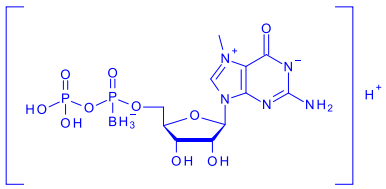
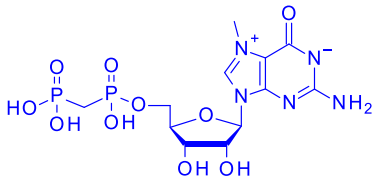
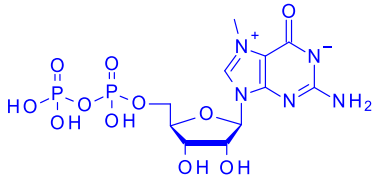
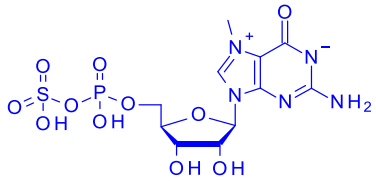
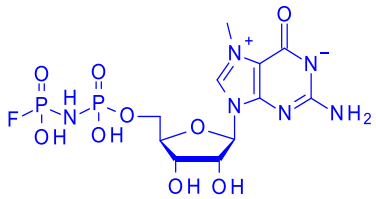
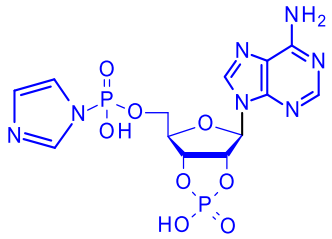
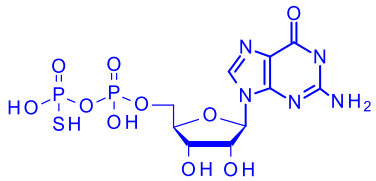
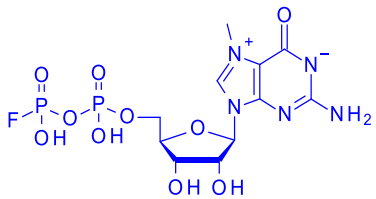
52.	$m^{2\,7,2'\text{-}O}GMPOCH_3$	N7,2'-O-dimethylguanosine 5′-(O-methyl)monophosphate		405	20	50	Experimental
53.	AMP-N6-etSH	N6-(2-mercaptoethyl)adenosine 5'-monophosphate		407	20	100	(Szczepaniak et al., 2012)
54.	m7GMPSCH3	N7-methylguanosine 5′-(S-methyl)monothiophosphate		407	20	50	Experimental
55.	cPAP	2’,3’-cyclophosphoadenosine 5’-phosphate		409	10	50	(Kowalska et al., 2012)
56.	2'-*O*-pentynyl AMP	2’-O- (5-pentynyl)adenosine 5’-monophosphate		413	100	100	(Jawalekar et al., 2008)
57.	GMP-Im	Guanosine 5’-phosphorimidazolide		413	20	50	(Jemielity et al., 2003)

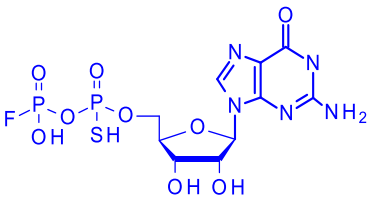
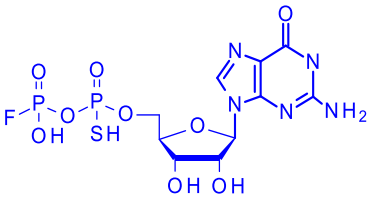
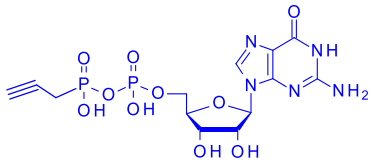
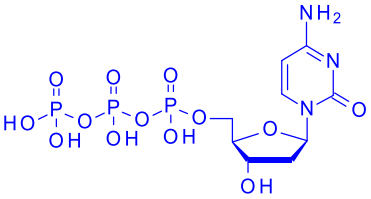
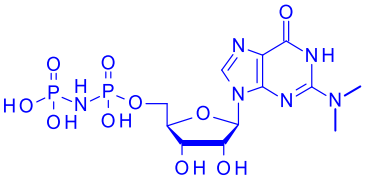
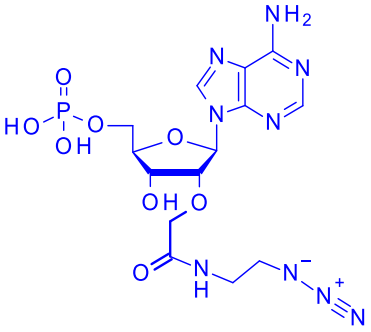
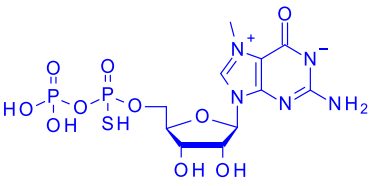
58.	$m^7GMPNH_2CH_2CCH$	N-propargyl N7-methylguanosine 5'-phosphoroamidate		414	50	50	(Walczak et al., 2017)
59.	AMP-N6-BDA	N6-(4-aminobutyl)adenosine 5'-monophosphate		418	20	100	(Szczepaniak et al., 2012)
60.	$ADP\alpha BH_3$ D1	Adenosine 5'-O-(1-boranodiphosphate)		425	10	100	(Strenkowska et al., 2012)
61.	$ADP\alpha BH_3$ D2	Adenosine 5'-O-(1-boranodiphosphate)		425	10	100	(Strenkowska et al., 2012)
62.	$ApCH_2p$	Adenosine 5'-O-methylenediphosphate		425	10	50	(Kalek et al., 2005)
63.	ApNHp	Adenosine 5'-O-imidodiphosphate		426	20	100	(Rydzik et al., 2012)
64.	m^7GMP-Im	N7-methylguanosine 5′-phosphorimidazolide		427	10	50	(Jemielity et al., 2003)
65.	ADP	Adenosine 5'-diphosphate		427	100	50	Commercial (Sigma Aldrich)

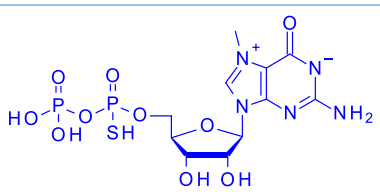
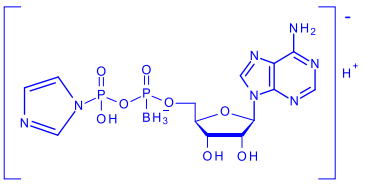
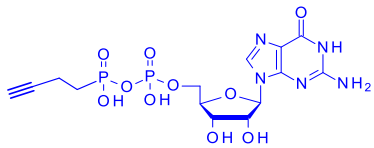
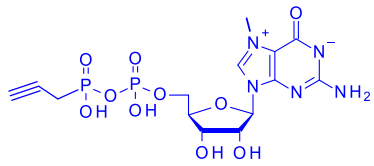
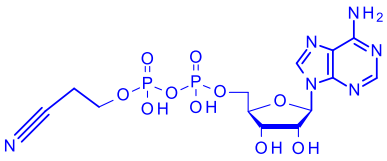
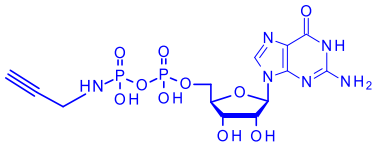
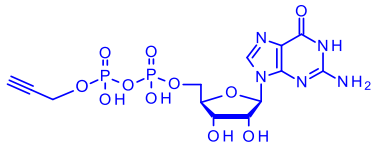
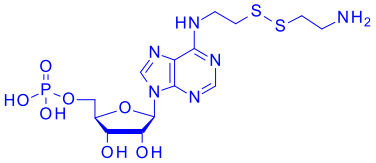
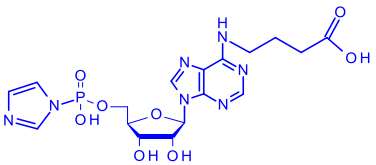
66.	APS	Adenosine 5'-phosphosulfate		427	100	50	(Kowalska et al., 2012)
67.	ApCH2pF	Adenosine 5′-(2-Fluoro-1,2-methylenediphosphate)		427	100	100	(Baranowski et al., 2015)
68.	2'-F 2'-dApCH$_2$p	2'-fluoro-2'-deoxyadenosine 5′-bisphosphonate		427	30	50	Experimental
69.	[^{18}O] ApCH2p	Adenosine 5'-O-(β[^{18}O]methylenediphosphate)		427	10	100	Experimental
70.	ADPF	Adenosine 5′-(2-fluorodiphosphate)		429	20	50	(Baranowski et al., 2015)
71.	2'-F-2'-d ADP	2'-fluoro-2'-deoxyadenosine 5′-(3-fluorodiphosphate)		429	20	100	Experimental
72.	GMPNHC$_2$H$_4$N$_3$	N-(2-azdioethyl) guanosine 5'-phosphoroamidate		431	20	100	(Walczak et al., 2017)
73.	GMPNH$_2$(CH$_2$)$_4$N$_3$	N-(4-aminobutyl) guanosine 5'-phosphoroamidate		433	50	70	(Walczak et al., 2017)

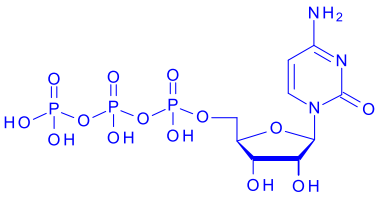
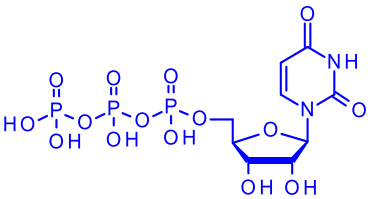
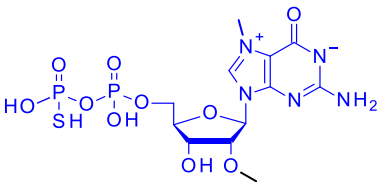
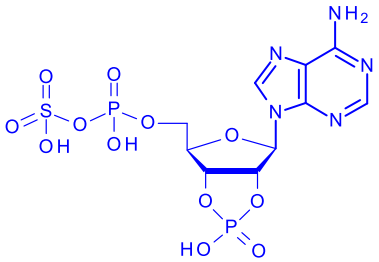
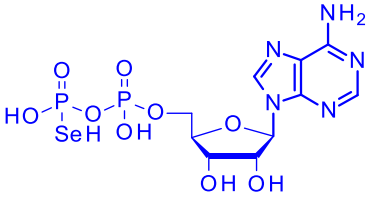
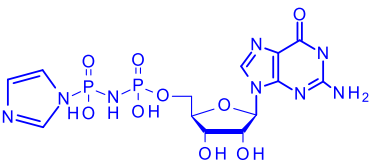
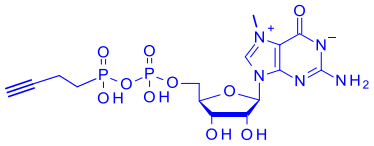
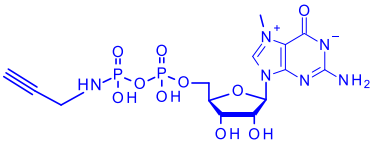
74.	GpCH$_2$p	Guanosine 5'-O-methylenediphosphate		441	10	100	(Kalek et al., 2006)
75.	m^{7}GppH	7-methylguanosine 5'-diphosphate		441	20	100	Experimental
76.	GpNHp	Guanosine 5'-O-imidodiphosphate		442	100	50	(TOMASZ et al., 1988)
77.	ADPαS	Adenosine 5'-(1-thiodiphosphate)		443	100	50	(Strenkowska et al., 2012)
78.	ADPβS	Adenosine 5'-(2-thiodiphosphate)		443	10	100	(Kowalska et al., 2007)
79.	GDP	Guanosine 5'-diphosphate		443	100	180	Commercial (Sigma Aldrich)
80.	GpBH$_3$pF_D1	Guanosine 5′-(2-Fluoro-1-boranodiphosphate)		443	10	100	(Baranowski et al., 2015)
81.	GpBH$_3$pF_D2	Guanosine 5′-(2-Fluoro-1-boranodiphosphate)		443	50	100	(Baranowski et al., 2015)

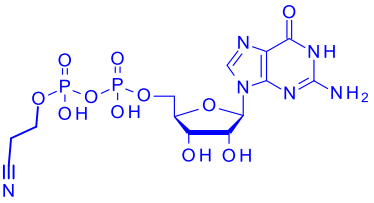
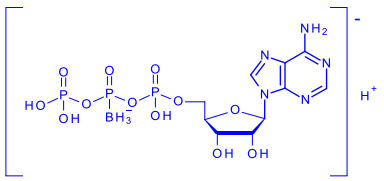
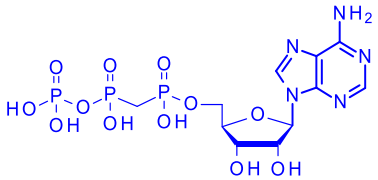
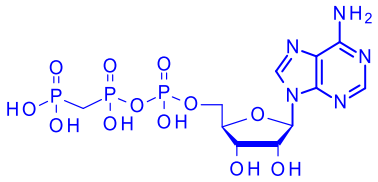
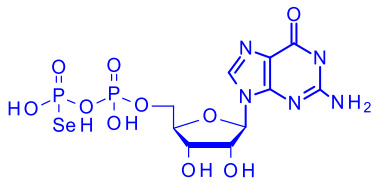
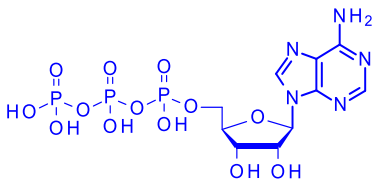
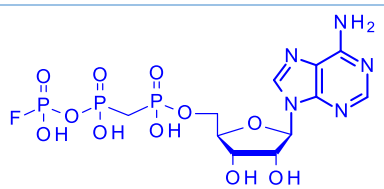
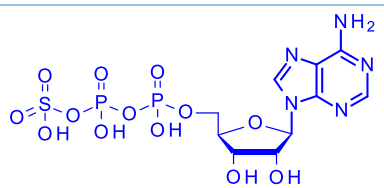
82.	GpCH2pF	Guanosine 5′-(2-Fluoro-1,2-methylenediphosphate)		443	10	50	(Baranowski et al., 2015)
83.	dGDPS	2-deoxyguanosine 5'-(2-thiodiphosphate)		443	30	50	Experimental + (Kowalska et al., 2007)
84.	GpNHpF	Guanosine 5′-(2-Fluoro-1,2-imidodiphosphate)		444	100	30	(Baranowski et al., 2015)
85.	GDPF	Guanosine 5′-Fluorodiphosphate		445	50	100	(Baranowski et al., 2015)
86.	[^{18}O]GDP	Guanosine 5′-(β[^{18}O]-diphosphate)		445	10	100	Experimental
87.	AMP-N6-HMDA	N6-(6-aminohexyl)adenosine 5'-monophosphate		446	10	50	(Szczepaniak et al., 2012)
88.	GppC$_2$H	β-C-(2-ethynyl) guanosine diphosphate		451	20	50	(Wanat et al., 2015)
89.	bn^{7}GMP	N7-benzylguanosine 5'-monophosphate		453	20	30	(Grudzien et al., 2004)

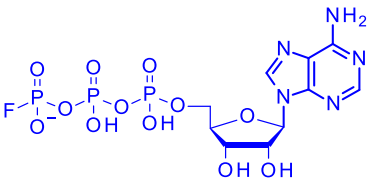
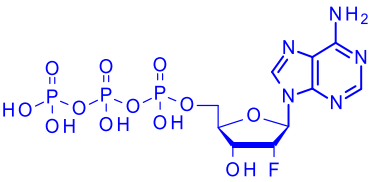
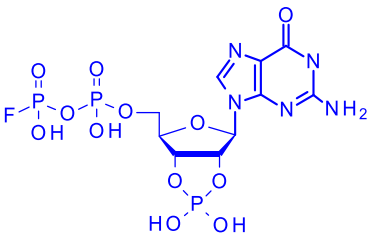
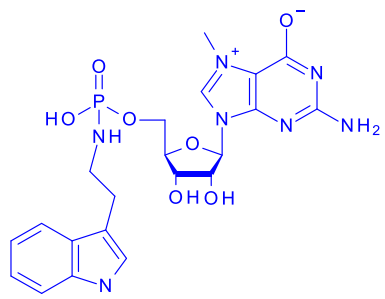
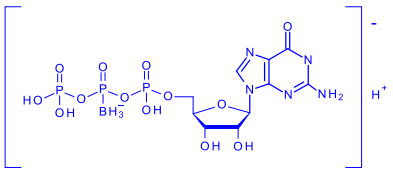
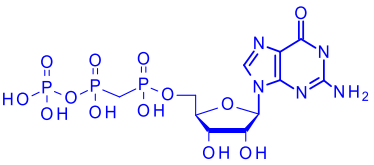
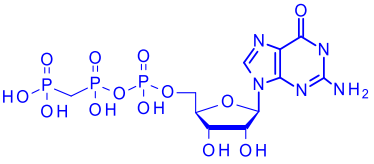
90.	$m^7GDP\alpha BH_3$	7-methylguanosine 5 -(1-boranodiphosphate)		455	10	100	(Kowalska et al., 2014)
91.	m^7GpCH_2p	7-methylguanosine 5′-methylenebis(phosphonate)		455	20	50	(Kalek et al., 2005)
92.	m^7GDP	7-methylguanosine 5'-diphosphate		457	10	30	(Jemielity et al., 2003)
93.	m^7GPS	7-methylguanosine 5'-phosphosulfate		457	100	100	(Kowalska et al., 2012)
94.	$m^7GpNHpF$	7-methylguanosine 5′-(2-fluoro-1,2-imidodiphosphate)		458	10	30	(Baranowski et al., 2015)
95.	cPAP-Im	2',3'-cyclophosphoadenosine 5'-phosphorimidazolide		459	10	100	(Kowalska et al., 2012)
96.	GDPβS	Guanosine 5′-(2-thiodiphosphate)		459	20	50	(Kowalska et al., 2007)
97.	m^7GDPF	7-methylguanosine 5′-fluorodiphosphate		459	10	50	(Baranowski et al., 2015)

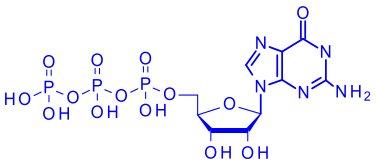
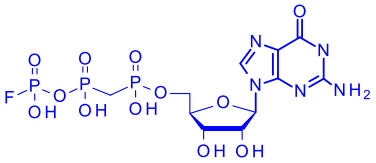
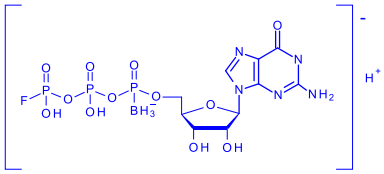
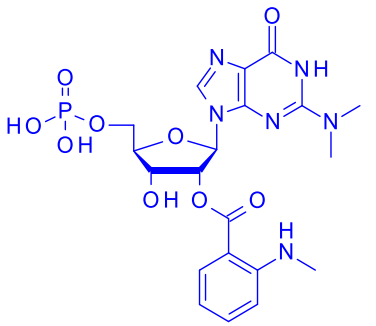
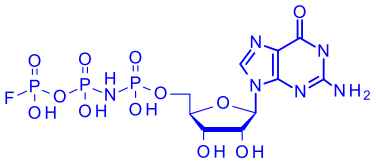
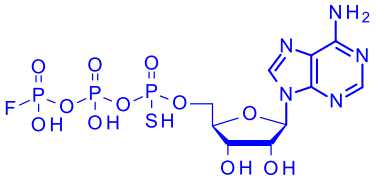
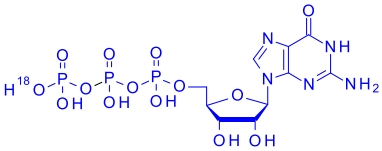
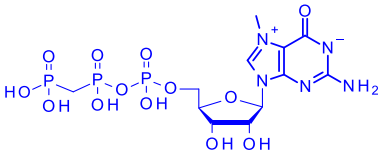
98.	GpSpF_D1	Guanosine 5′-(2-Fluoro-1-thiodiphosphate)		461	100	100	(Baranowski et al., 2015)
99.	GpSpF_D2	Guanosine 5′-(2-Fluoro-1-thiodiphosphate)		461	100	100	(Baranowski et al., 2015)
100	$GppC_3H_3$	β-C-(2-propargyl) guanosine diphosphate		465	10	100	(Wanat et al., 2015)
101	2'-dCTP	2’deoxycytidine 5′-triphosphate		467	20	150	Commercial (Sigma Aldrich)
102	DMGpNHp	*N,N*-dimethylguanosine 5’-imidodiphosphate		470	10	30	(Zytek et al., 2014)
103	2’-*O*-MCE-N_3 AMP-	2’-O-(*N*-(2-Azidoethyl)carbamoyl) methyladenosine 5’-monophosphate		473	20	50	(Wojtczak et al., 2016)
104	m^7GDPαS D1	N 7 -methylguanosine 5’-O-(1-thiodiphosphate)		473	10	50	(Strenkowska et al., 2012)

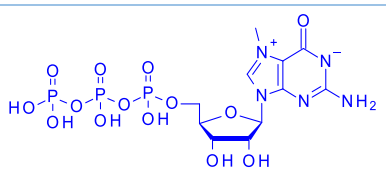
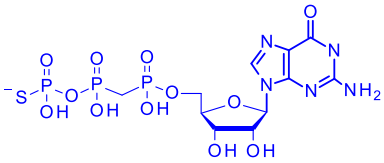
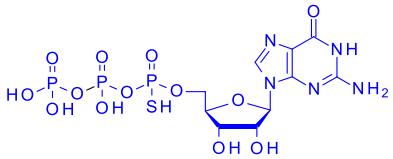
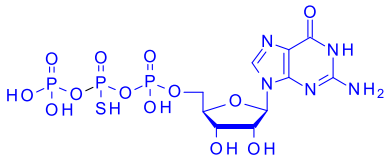
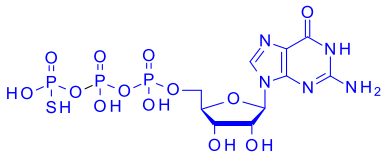
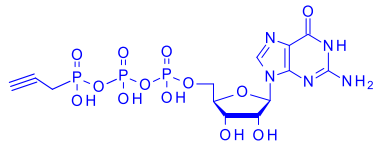
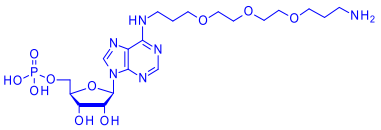
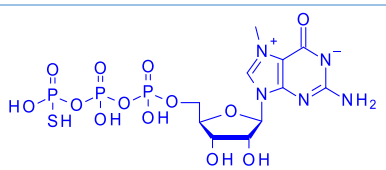
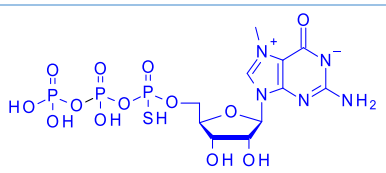
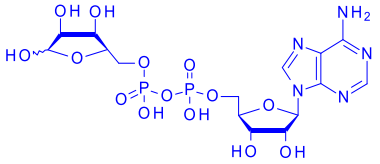
105	$m^7GDP\alpha S$ D2	N 7 -methylguanosine 5'-O-(1-thiodiphosphate)		473	10	50	(Strenkowska et al., 2012)
106	$ApBH_3pIm$	Adenosine 5 -(1-borano-2-imidazoyl-diphosphate),		475	100	100	(Kowalska et al., 2014)
107	$GppC_4H_5$	guanosine 5'- [2-C-(3-butynyl)diphosphate]		479	20	100	(Wanat et al., 2015)
108	m7GppC3H3	β-C-(2-propargyl) 7-methylguanosine diphosphate		479	10	100	(Walczak et al., 2017)
109	AppEtCN	Adenosine 5′-[(2-O-cyanoethyl)diphosphate]		480	50	50	(Strenkowska et al., 2012)
110	GppNHC3H3	Guanosine 5′-(N2-propargyl-2-amino)diphosphate		480	20	50	(Walczak et al., 2017)
111	GppOC3H3	Guanosine 5′-(2-propargyl)diphosphate		481	20	50	(Walczak et al., 2017)
112	AMP-NH-etS-S-etNH2	N6-cystamine-adenosine 5'-monophosphate		482	20	70	(Szczepaniak et al., 2012)
113	AMPIm-6-GABA	N6-(4-carboxybutyl)-adenosine 5'-monophosphate		483	50	100	(Szczepaniak et al., 2012)

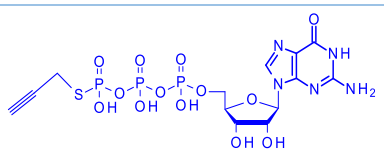
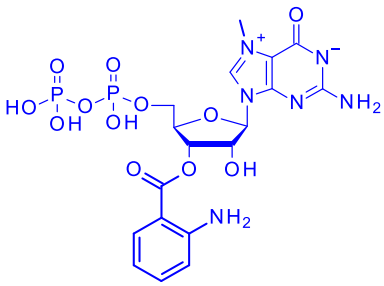
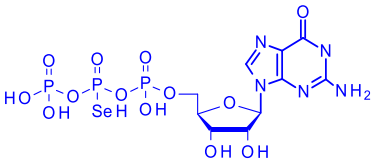
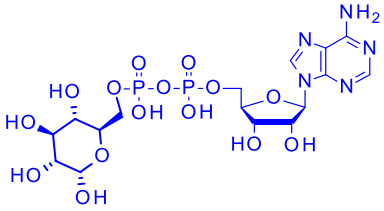
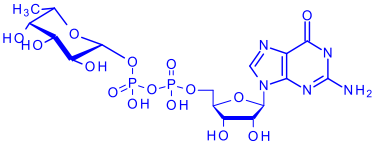
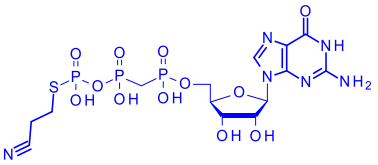
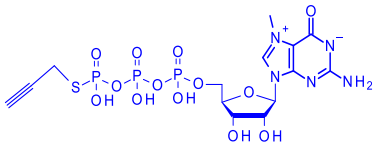
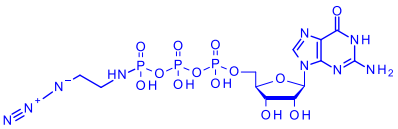
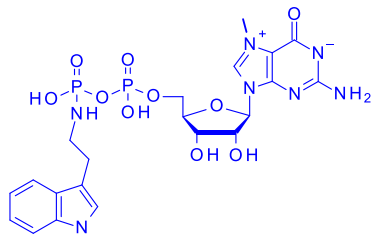
114	CTP	Cytidine 5'-triphosphate		483	20	100	Commercial (Sigma Aldrich)
115	UTP	Uridine 5′-triphosphate		484	20	100	Commercial (Sigma Aldrich)
116	$m^{7,2}$GDPβS	N7,2'-O-dimethylguanosine 5′-(2-thiodiphosphate)		487	100	30	(Kowalska et al., 2007)
117	cPAPS	2',3'-cyclophosphoadenosine 5'-phosphosulfate		489	100	100	(Kowalska et al., 2012)
118	ADPβSe	Adenosine 5′-(2-selenodiphosphate)		491	10	50	(Kowalska et al., 2009)
119	GpNHpIm	Guanosine 5′-(2-Imidazoyl-1,2-imidodiphosphate)		492	30	30	(Baranowski et al., 2015)
120	m7GppC4H5	β-C-(3-butynyl) 7-methylguanosine diphosphate		493	10	100	(Walczak et al., 2017)
121	m7GppNHC3H3	7-methylguanosine 5′-(N2-propargyl-2-amino)diphosphate		494	20	30	(Walczak et al., 2017)

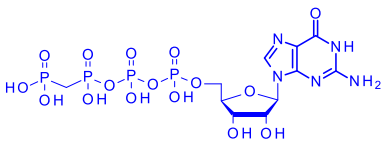
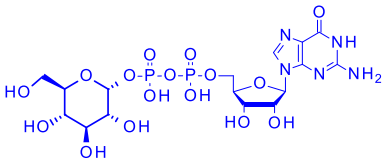
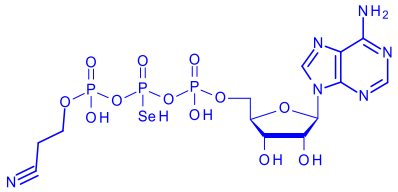
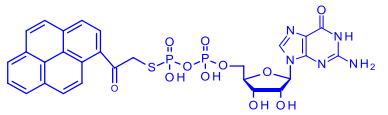
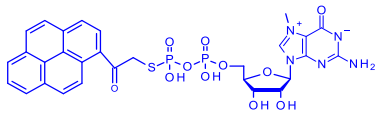
122	GDP-EtCN	Guanosine 5′-[(2-O-cyanoethyl)diphosphate]		496	20	100	(Strenkowska et al., 2012)
123	ATPβBH3	Adenosine 5’-O-(2-boranotriphosphate)		505	100	100	(Strenkowska et al., 2012)
124	ApCH2pp	Adenosine 5’-O-(1,2-methylenetriphoshate)		505	20	100	(Spelta et al., 2003)
125	AppCH2p	Adenosine 5’-O-(2,3-methylenetriphoshate)		505	100	50	(Guranowski et al., 2006)
126	GDPβSe	Guanosine 5′-(2-selenodiphosphate)		507	10	100	(Kowalska et al., 2009)
127	ATP	Adenosine 5’-triphosphate		507	20	100	Commercial (Sigma Aldrich)
128	$ApCH_2ppF$	Adenosine 5′-(3-Fluoro-1,2-methylenetriphosphate)		507	20	100	(Baranowski et al., 2015)
129	APPS	adenosine 5'-diphosphosulfate		507	20	100	(Kowalska et al., 2012)

130	ATPF	Adenosine 5′-(3-Fluorotriphosphate)		509	20	50	(Baranowski et al., 2015)
131	2’-F-2’-d ATP	2’-fluoro-2’-deoxyadenosine 5′-(3-Fluorotriphosphate)		509	100	100	Experimental
132	cPADPF	2′,3′-Cyclic-phosphoadenosine 5′-(2-Fluorodiphosphate		509	100	100	(Baranowski et al., 2015)
133	m7GMPTA	7-methylguanosine 5’-(tryptaminophosphate)		519	20	50	(Guranowski et al., 2011)
134	GTPβBH_3	Guanosine 5’-O-(2-boranotriphosphate)		521	10	50	(Strenkowska et al., 2012)
135	GpCH_2pp	Guanosine 5’-O-(1,2-methylenetriphoshate)		521	10	50	(Strenkowska et al., 2012)
136	GppCH_2p	Guanosine 5’-O-(2,3-methylenetriphoshate)		521	10	150	(Rydzik et al., 2009)

137	GTP	Guanosine 5′-triphosphate		523	100	100	Commercial (Sigma Aldrich)
138	GpCH$_2$ppF	Guanosine 5′-(2-Fluoro-1,2-methylenediphosphate)		523	20	50	(Baranowski et al., 2015)
139	GpBH$_3$ppF	Guanosine 5′-(2-Fluoro-1-boranodiphosphate)		523	10	100	(Baranowski et al., 2015)
140	3’ (2’) Mant-GMP	2’-/3’-Methylanthraniloyl guanosine 5’-monophosphate		524	100	100	(Ziemniak et al., 2013)
141	GpNHppF	Guanosine 5′-(3-Fluoroimidotriphosphate)		524	20	50	(Baranowski et al., 2015)
142	ApSppF	Adenosine 5′-(3-Fluoro-1-thiotriphosphate)		525	100	50	(Baranowski et al., 2015)
143	[^{18}O]GTP	Guanosine 5′-(γ[^{18}O]triphosphate)		525	10	100	Experimental
144	m7GppCH2p	7-methylguanosine 5’-O-(2,3-methylenetriphoshate)		535	10	100	(Guranowski et al., 2006)

145	m^7GTP	7-methylguanosine 5'-triphosphate		537	50	100	(Jemielity et al., 2003)
146	GpCH2ppS	Guanosine 5'-(1,2-methylene-3-thiotriphosphate)		537	20	30	(Strenkowska et al., 2012)
147	GTPαS	Guanosine 5'-(1-thiotriphosphate)		539	10	80	(Strenkowska et al., 2010)
148	GTPβS	Guanosine 5'-(2-thiotriphosphate)		539	10	80	(Strenkowska et al., 2012)
149	GTPγS	Guanosine 5'-(3-thiotriphosphate)		539	10	80	(Kowalska et al., 2007)
150	GpppC3H3	Guanosine 5'- [β-C-(2-propargyl)triphosphate]		545	20	100	(Wanat et al., 2015)
151	AMP-N6-TTDA	13-amino-4,7,10-Trioxa-decane-N6-adenosine 5'-monophosphate		550	10	50	(Szczepaniak et al., 2012)
152	$m^7GTPγS$	7-methylguanosine 5'-(3-thiotriphosphate)		553	100	30	(Kowalska et al., 2007)
153	m7GTPαS_D1	7-methylguanosine 5'-(1-thiotriphosphate)		553	10	100	(Kowalska et al., 2009)
154	ADP-5-Rib	Adenosine Diphosphate 5-D-Ribofuranose		559	10	100	(Dabrowski-Tumanski et al., 2013)

155	GpppSC3H3	S-(2-propargyl) guanosine 5'-(3-thiotriphosphate)		577	20	70	(Walczak et al., 2017)
156	Ant-m7 GDP	2'-/3'- Anthraniloyl 7-methylguanosine 5'-monophosphate		579	100	100	(Ziemniak et al., 2013)
157	GTPβSe	Guanosine 5'-O-(2-selenotriphosphate)		587	100	30	(Strenkowska et al., 2012)
158	ADP-6-Glc	Adenosine Diphosphate 6-D-Glucopyranose		589	50	30	(Dabrowski-Tumanski et al., 2013)
159	GDP-1-β-Fuc	Guanosine Diphosphate 1-β-L-Fucopyranose		589	10	30	(Dabrowski-Tumanski et al., 2013)
160	GpCH2ppSEtCN	S-(2-cyanoethyl) guanosine 5'-(1,2-methylene-3-thio-triphosphate)		590	100	100	(Strenkowska et al., 2012)
161	$m^7GpppSC_3H_3$	S-(2-propargyl) 7-methylguanosine 5'-(3-thiotriphosphate)		591	10	100	(Walczak et al., 2017)
162	$GpppNHC_2H_4N_3$	N-(2-propargyl) guanosine 5'-(3-aminotriphosphate)		591	20	100	(Walczak et al., 2017)
163	m7GDPβTA	7-methylguanosine 5'-(2-tryptaminodiphosphate)		599	20	50	(Guranowski et al., 2011)

164	GpppCH2p	Guanosine 5'-O-(3,4-methylenetetraphoshate)		601	10	70	(Guranowski et al., 2006)
165	GDP-1-α-Glc	Guanosine 5-Diphosphate 1-α-D-Glucopyranose		605	20	100	(Dabrowski-Tumanski et al., 2013)
166	AppSepEtCN	adenosine 5'-[2-seleno-3-(2-O-cyanoetylo)triphosphate]		624	10	100	(Strenkowska et al., 2012)
167	GDPβS–AcPy	S-(1-acetylpyrenyl) guanosine 5'-(2-thiodiphosphate)		701	50	50	(Kasprzyk et al., 2016)
168	m^7 GDPβS–AcPy	S-(1-acetylpyrenyl) 7-methylguanosine 5'-(2-thiodiphosphate)		715	10	100	(Kasprzyk et al., 2016)

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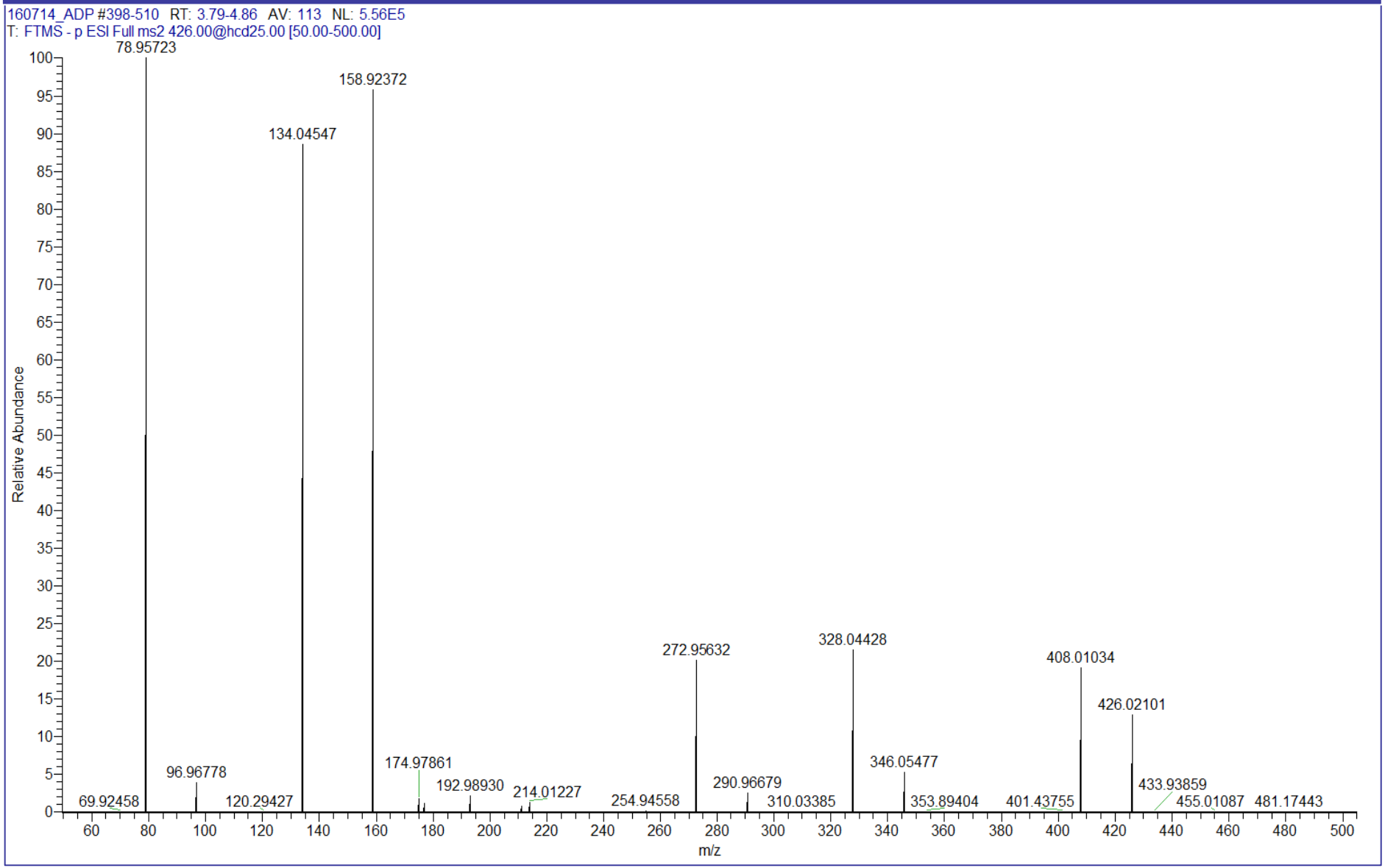
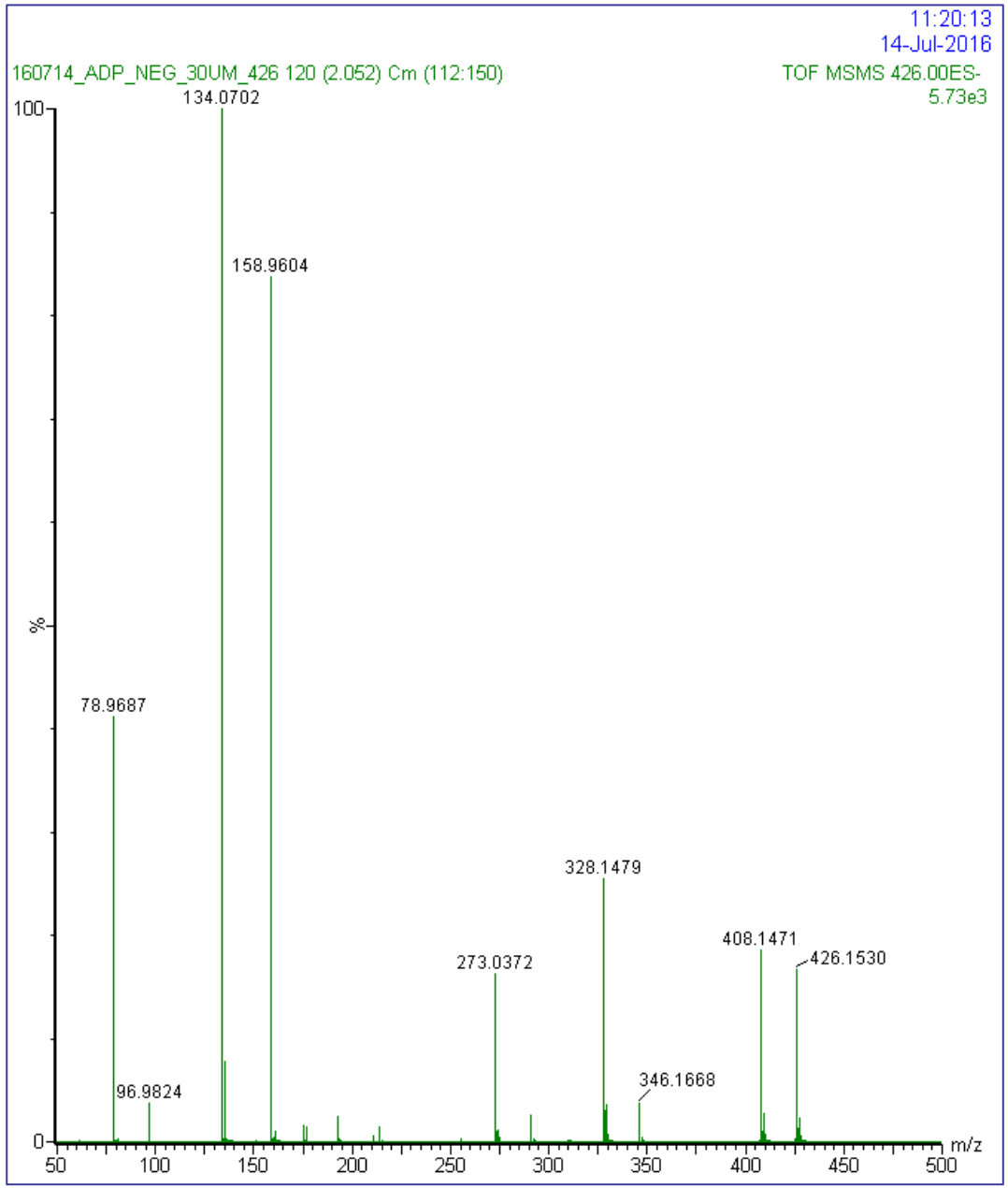
Appendix A - AMP and ADP spectra on different equipment types

Equipment	ADP 30 µM ($MetOH:H_2O$ 1:1, 5% NH_3)		AMP 30 µM ($MetOH:H_2O$ 1:1, 5% NH_3)	
QexactiveThermo	ESI negative ion mode	ESI positive ion mode	ESI negative ion mode	ESI positive ion mode
QTOF Waters				
QTRAP 3200 Sciex Q1				
QTRAP 3200 Sciex EPI (Enhance Product Ion)				
API 3200 AB Sciex Q1				

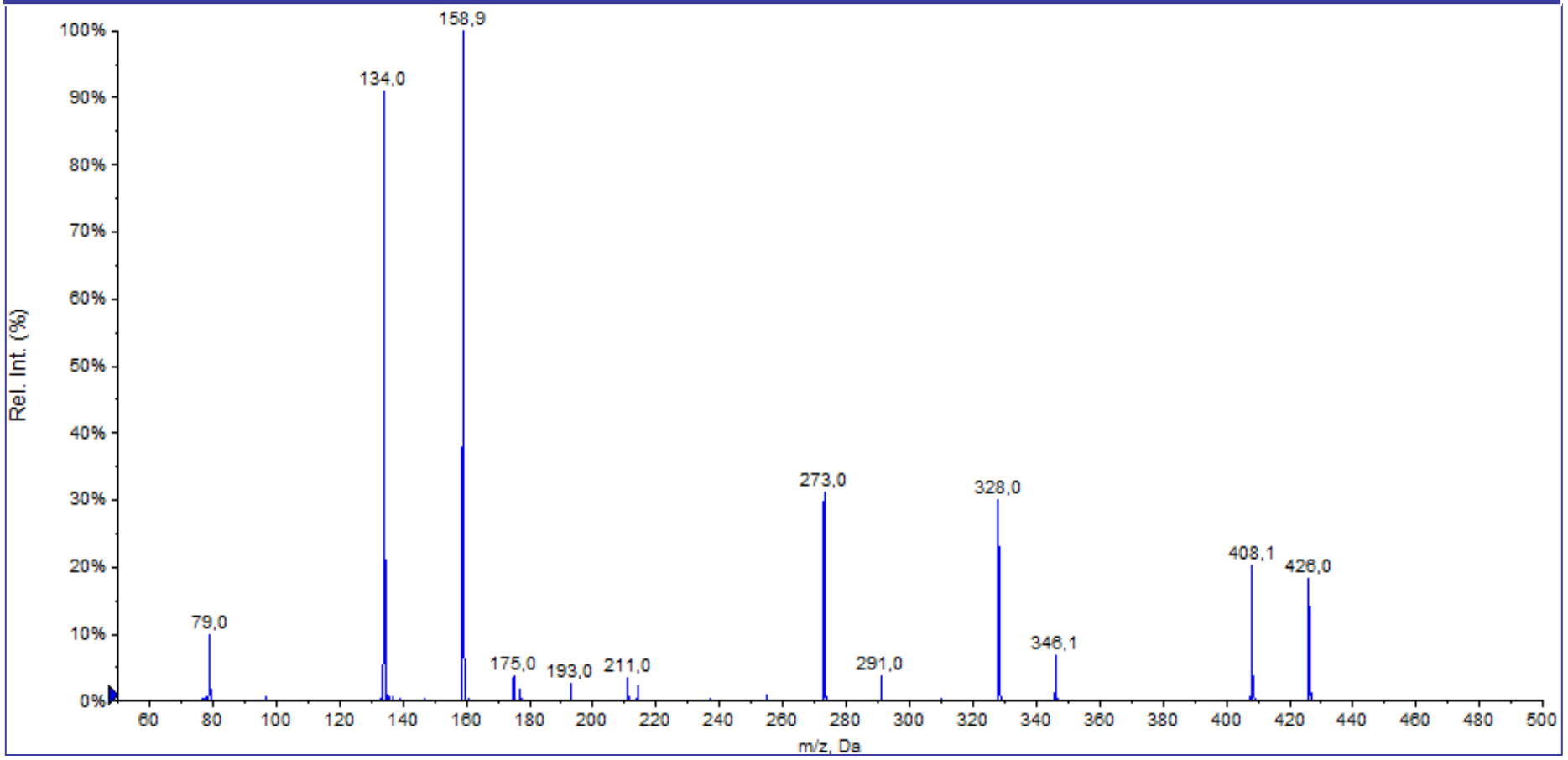
QExactive Thermo, ADP, CE 25 ESI (-)



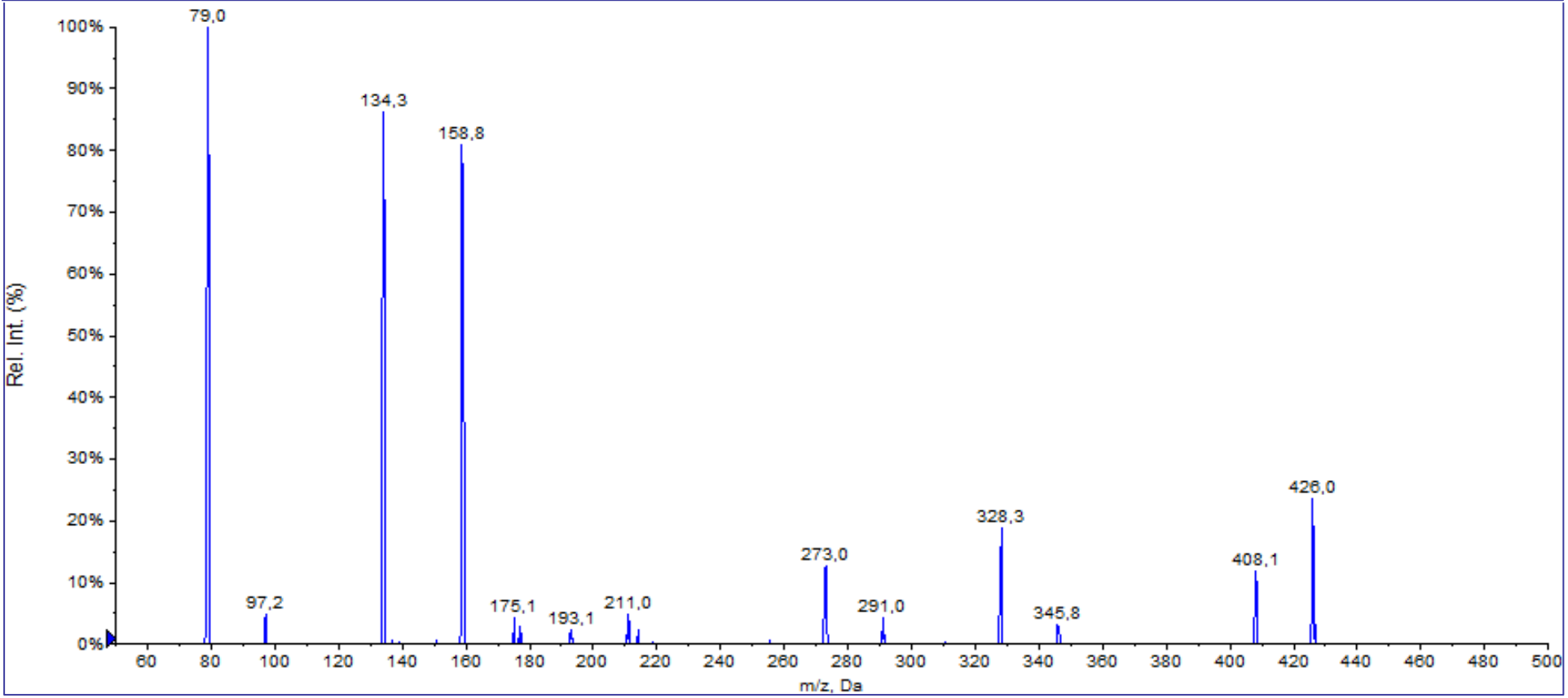
QTOF Waters, ADP, CE 20 ESI (-)



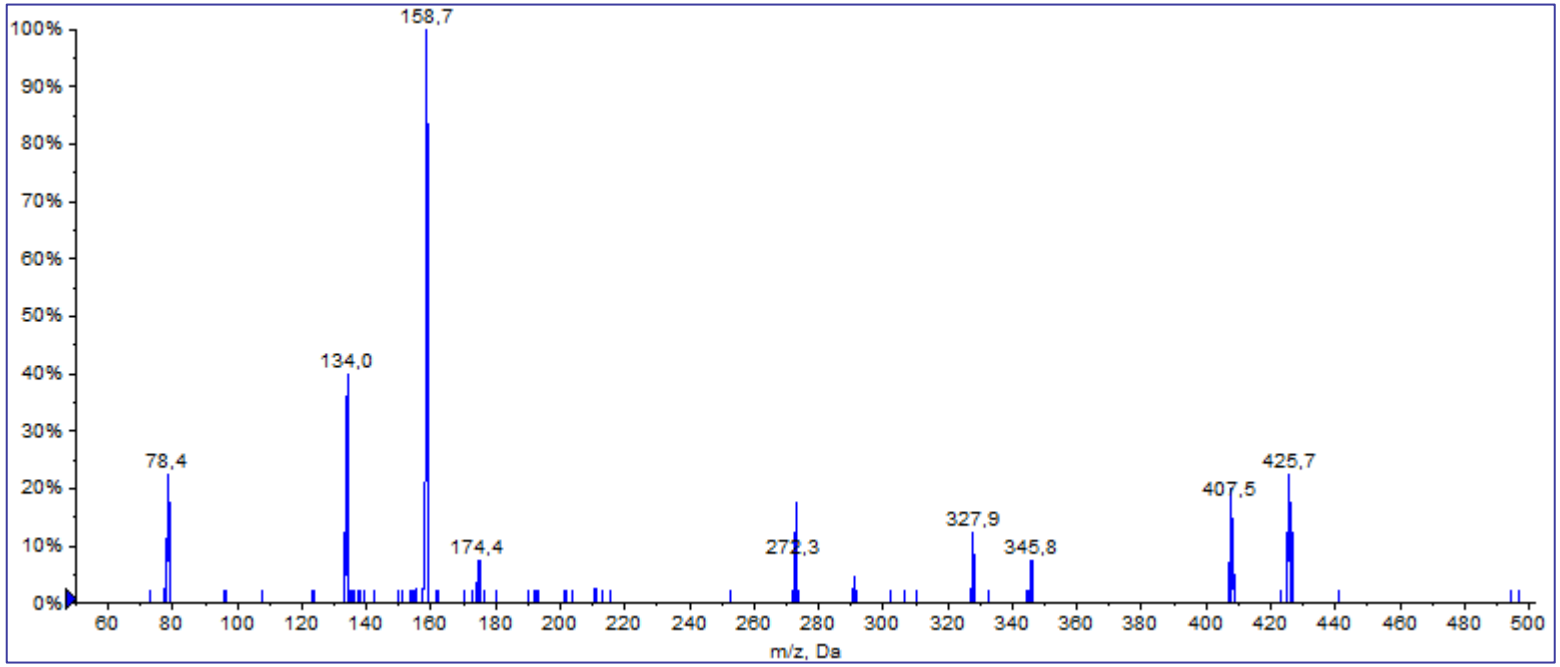
QTRAP 3200 AB Sciex, ADP, CE 39 ESI (-), EPI



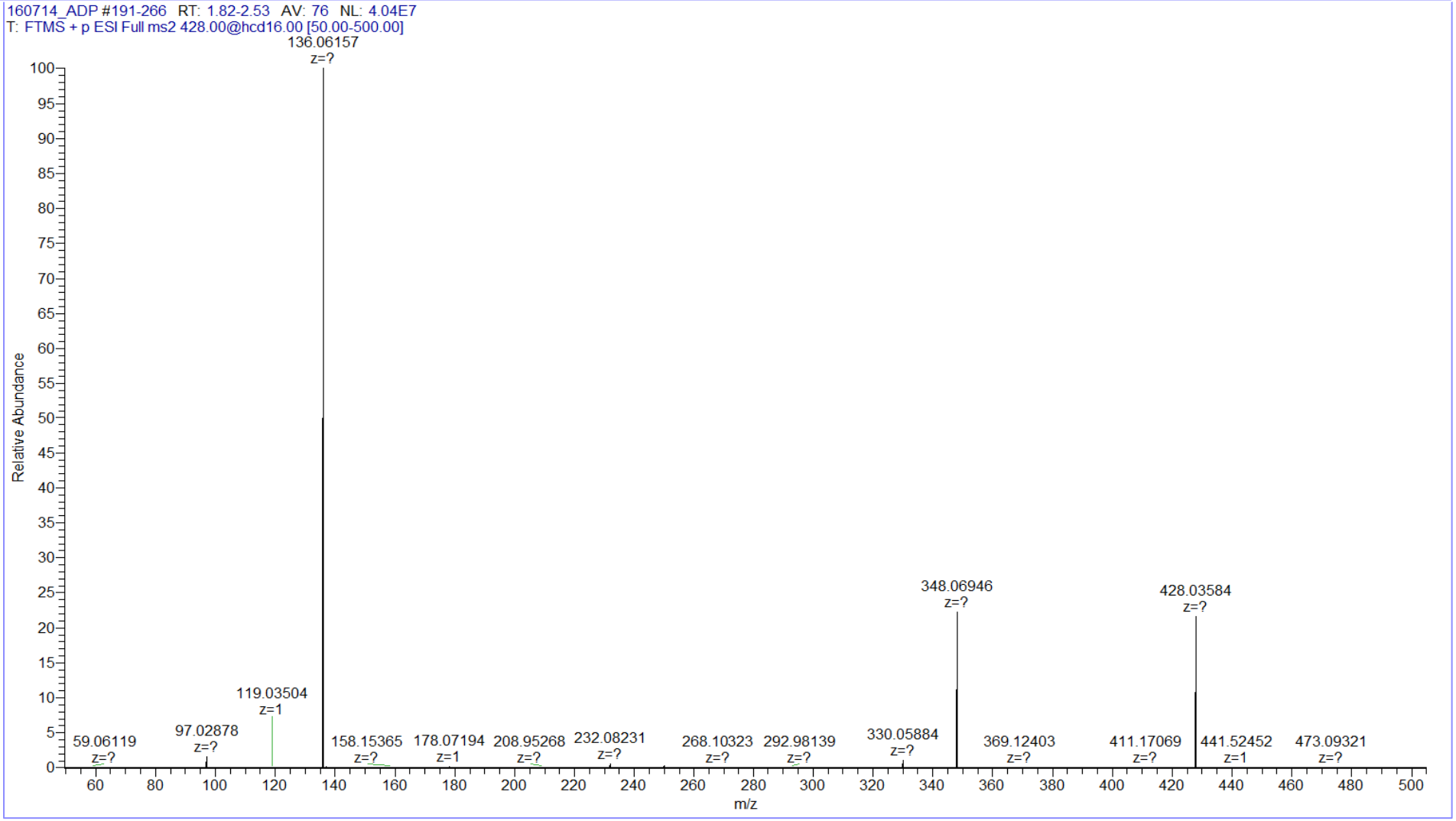
QTRAP 3200 AB Sciex, ADP, CE 40 ESI (-), Q1/Q3



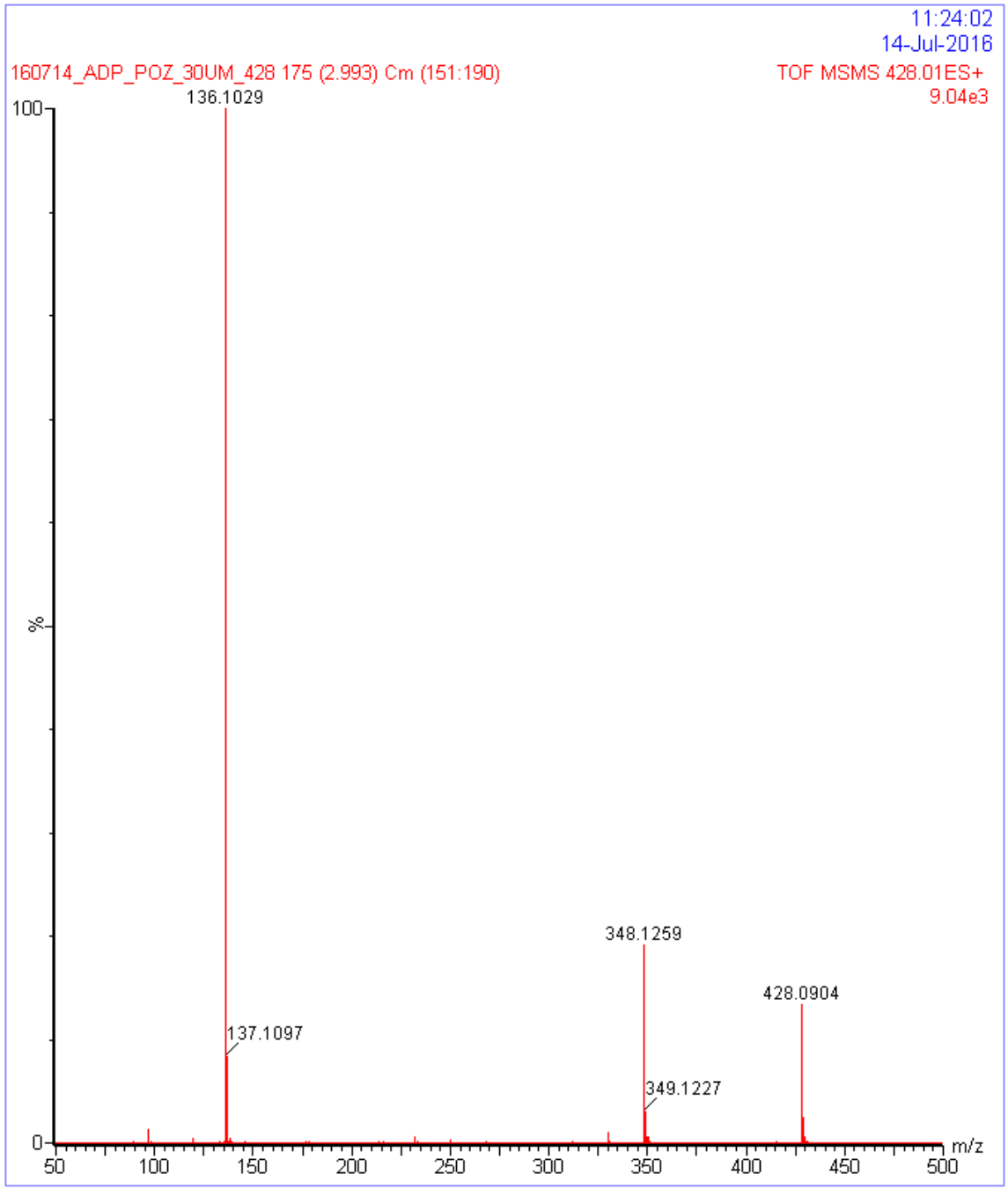
API 3200 AB Sciex, ADP, CE 45 ESI (-)

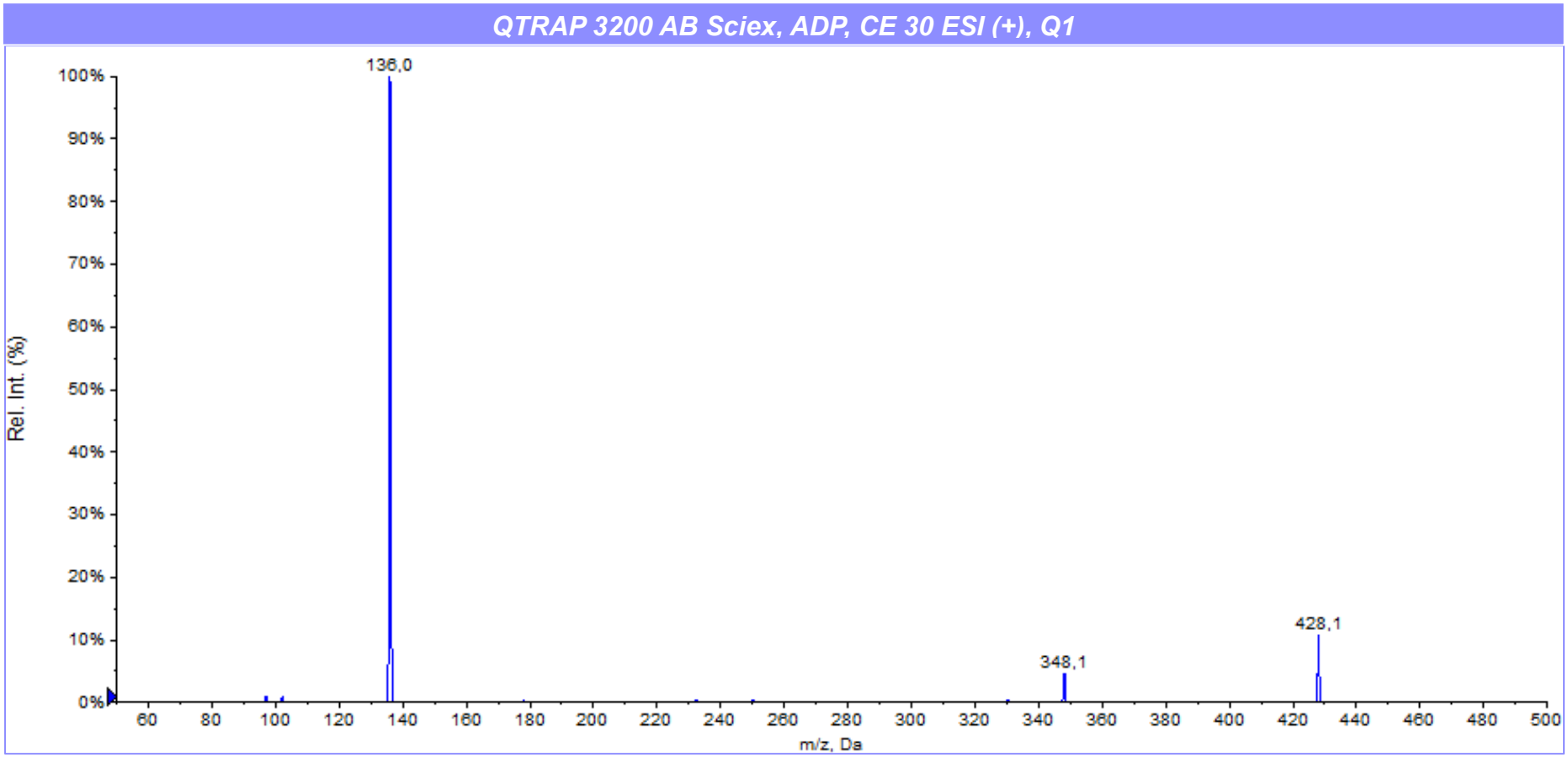


QExactive Thermo, ADP, CE 16 ESI (+)

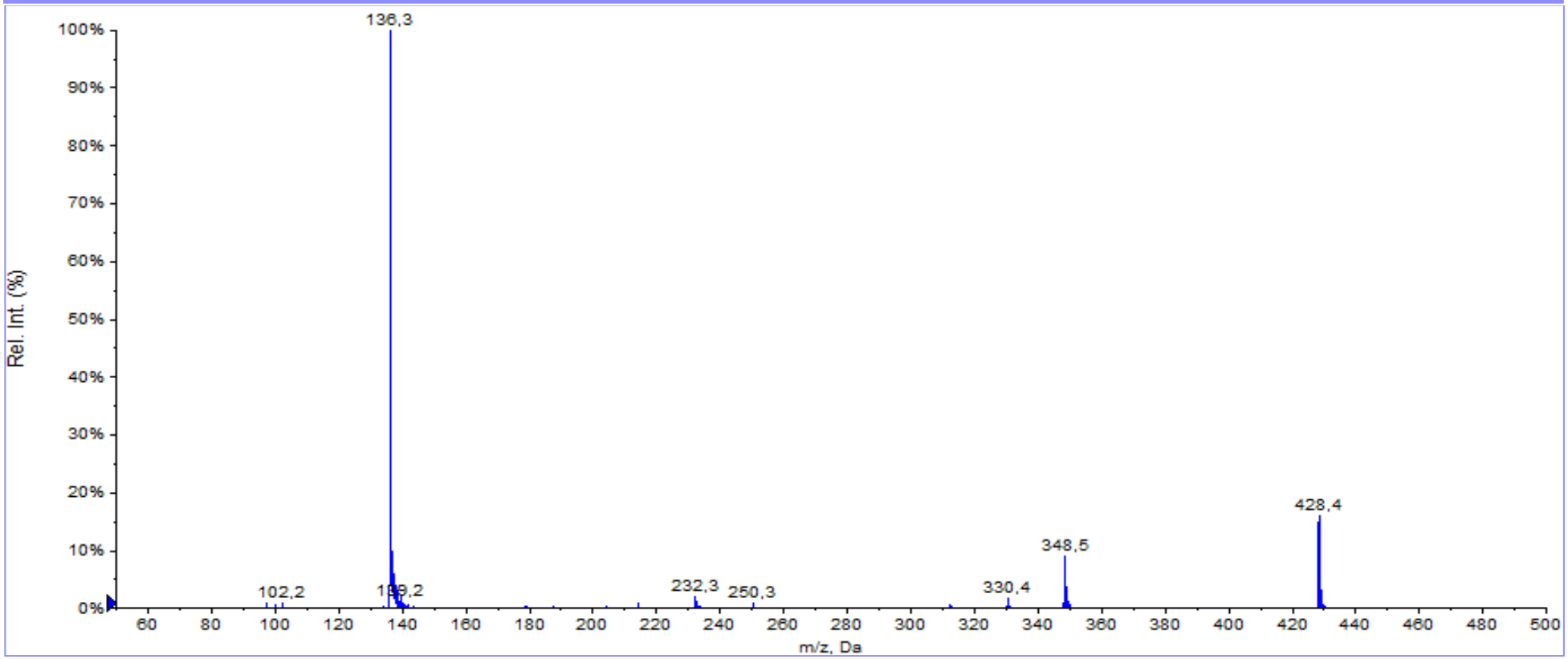


QTOF Waters, ADP, CE 13 ESI (+)

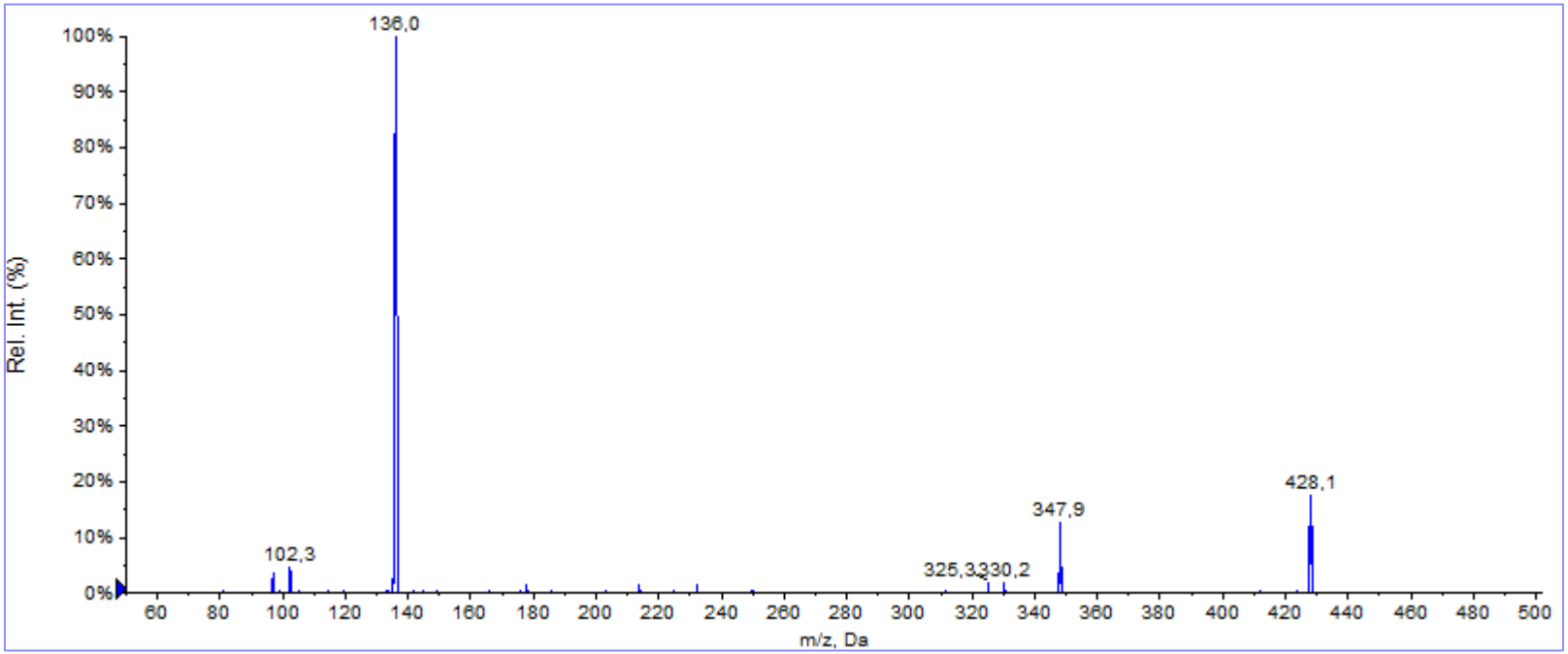



QTRAP 3200 AB Sciex, ADP, CE 30 ESI (+), Q1
136,0
348,1
428,1
Rel. Int. (%)
m/z, Da
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500

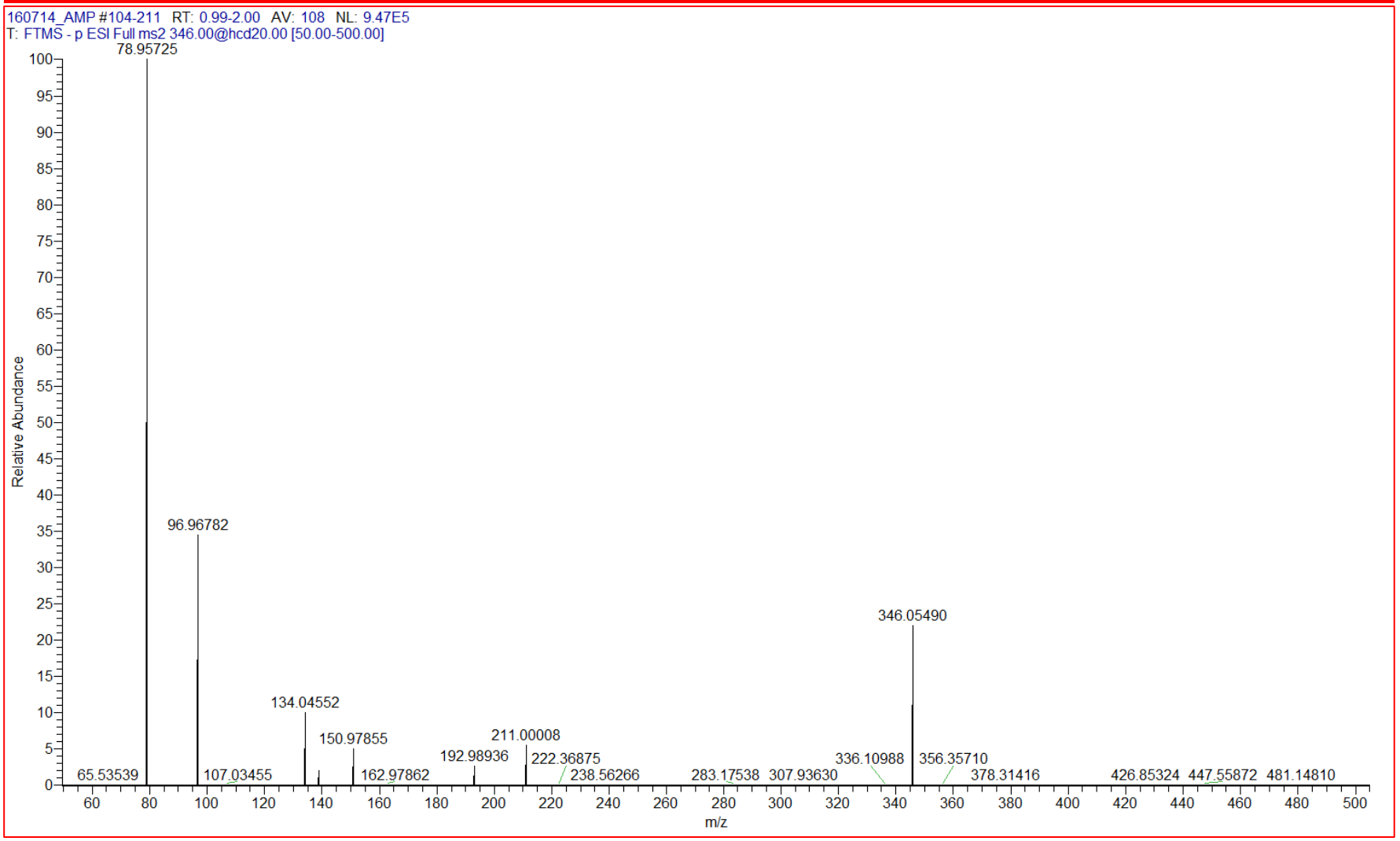
QTRAP 3200 AB Sciex. ADP. CE 30 ESI (+). EPI



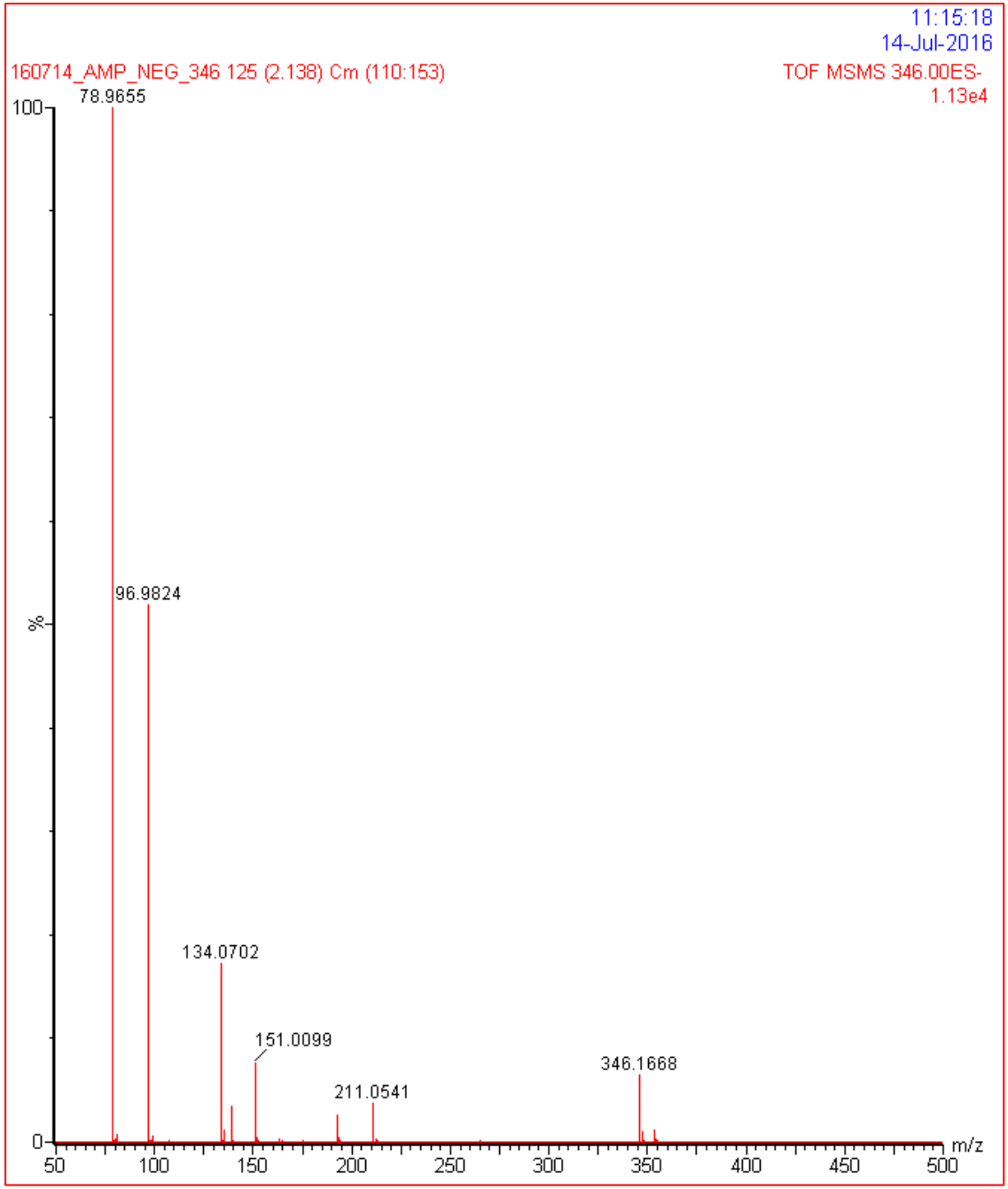
API 3200 AB Sciex, ADP, CE 30 ESI (+)



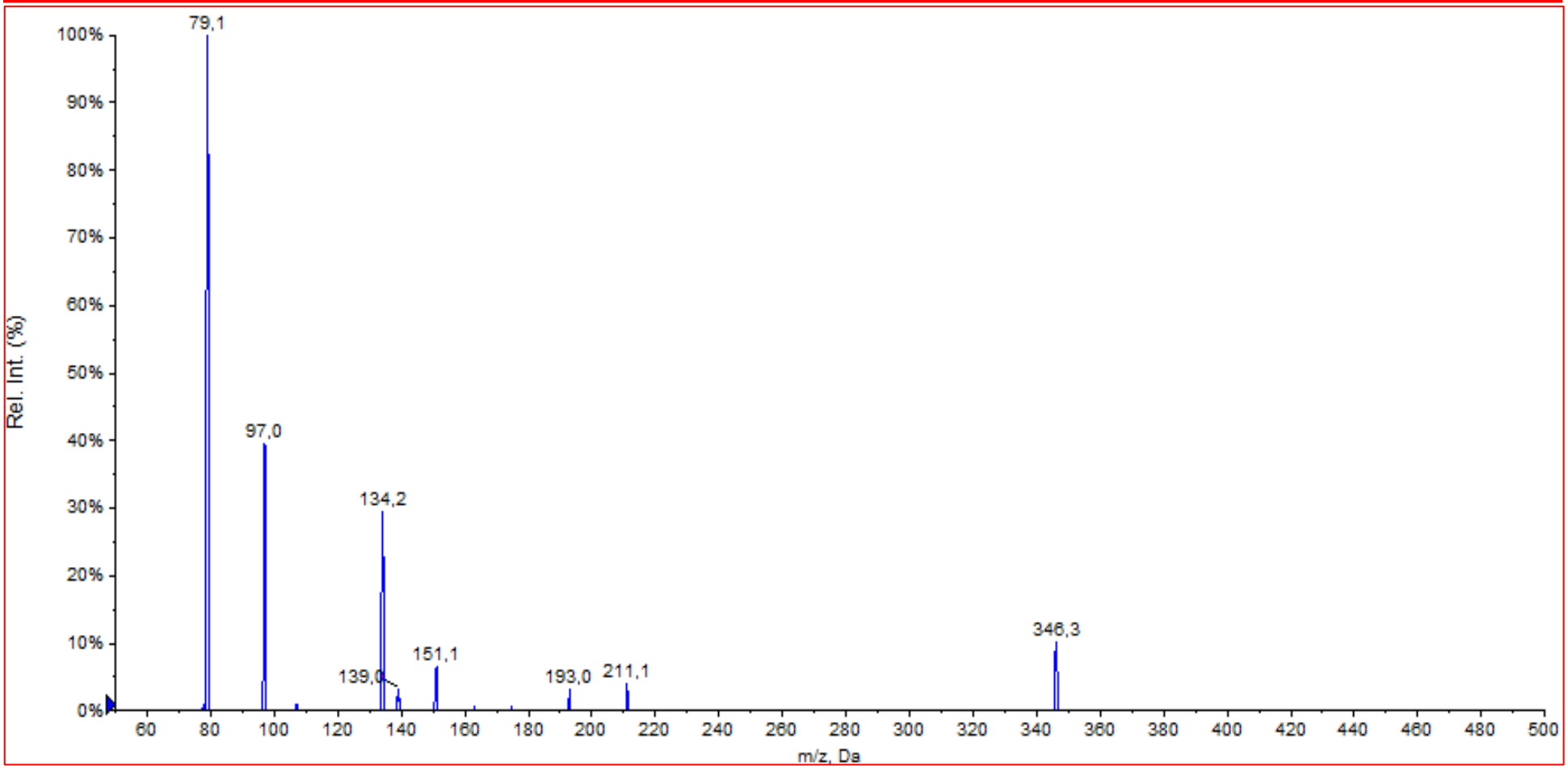
QExactive Thermo, AMP, CE 20 ESI (-)



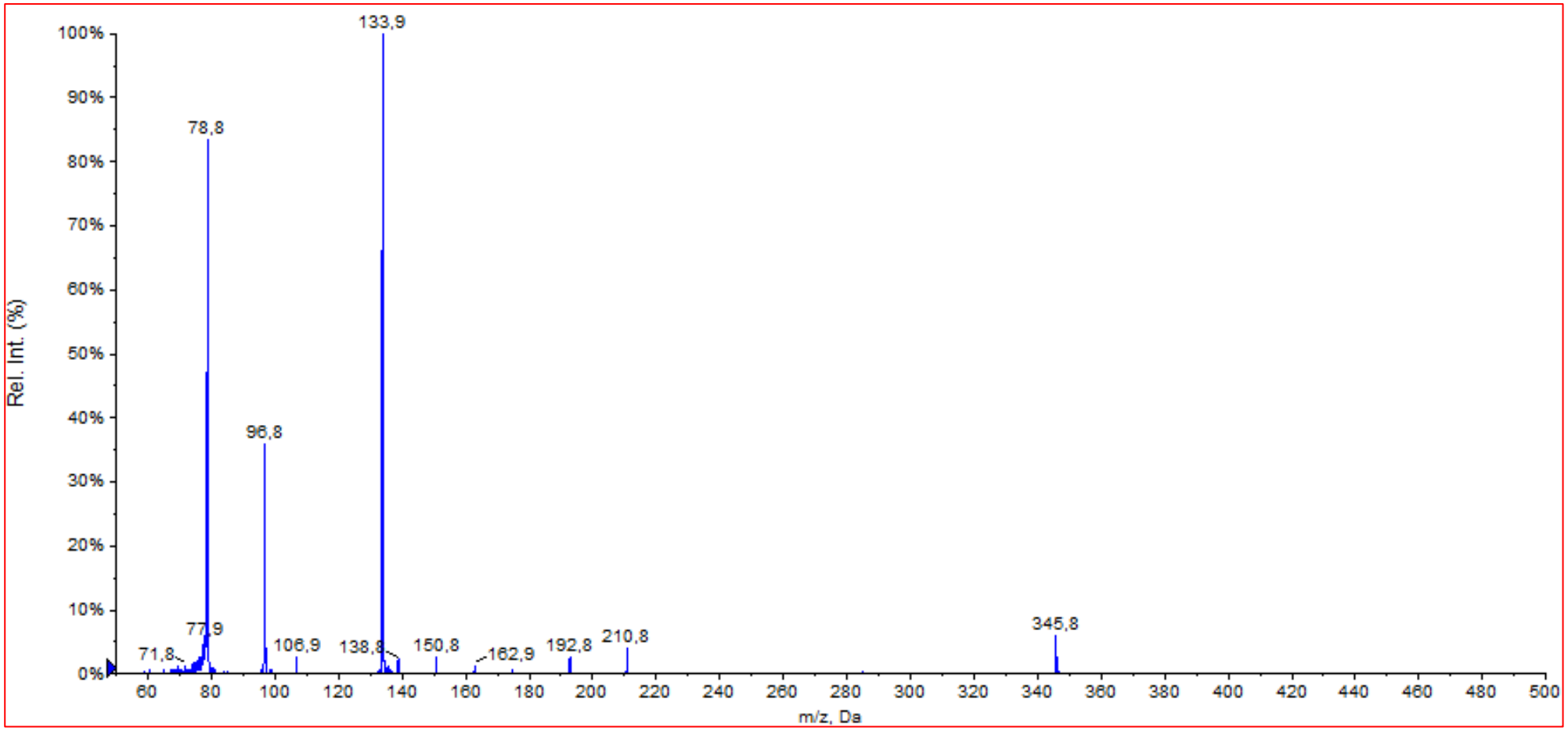
QTOF Waters, AMP, CE 20 ESI (-)



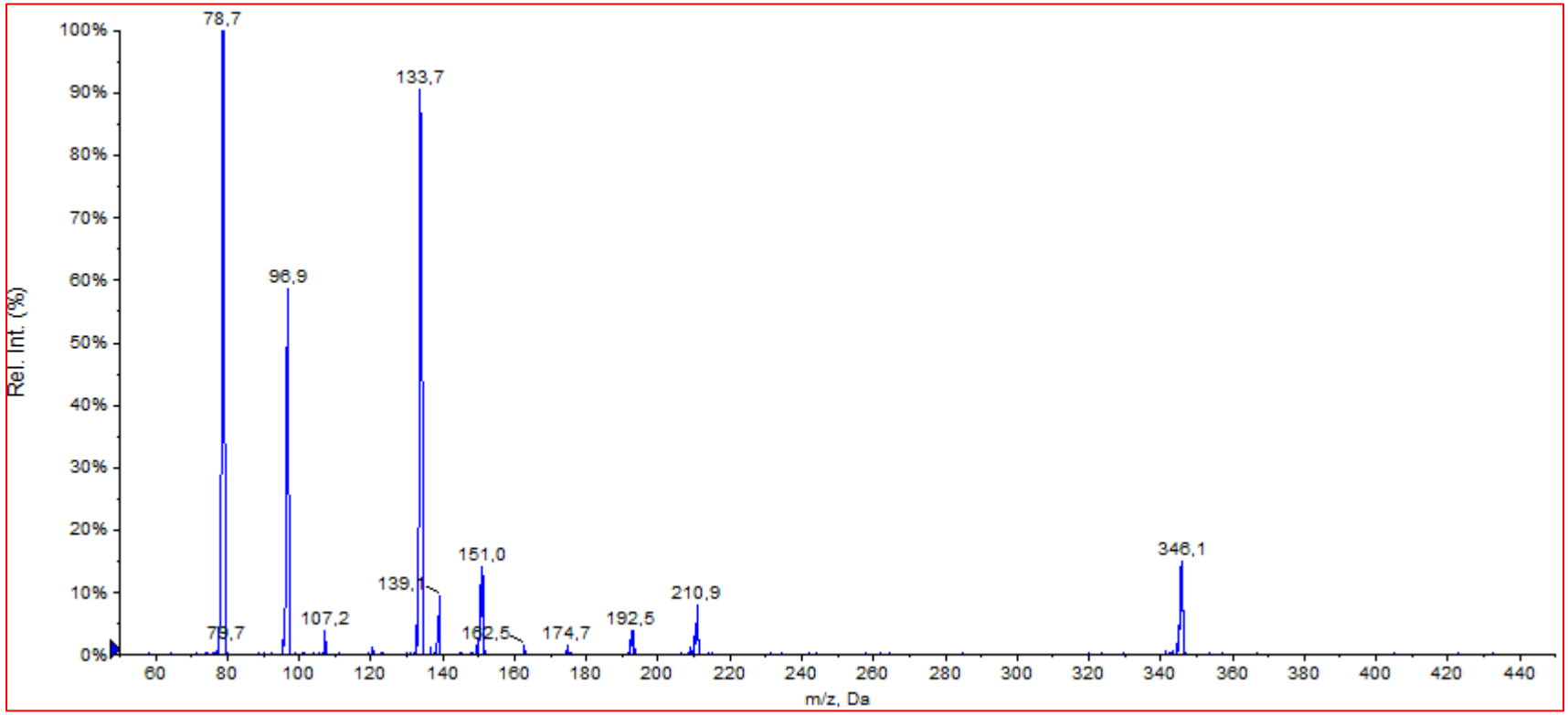
QTRAP 3200AB Sciex, AMP, CE 39 ESI (-), Q1



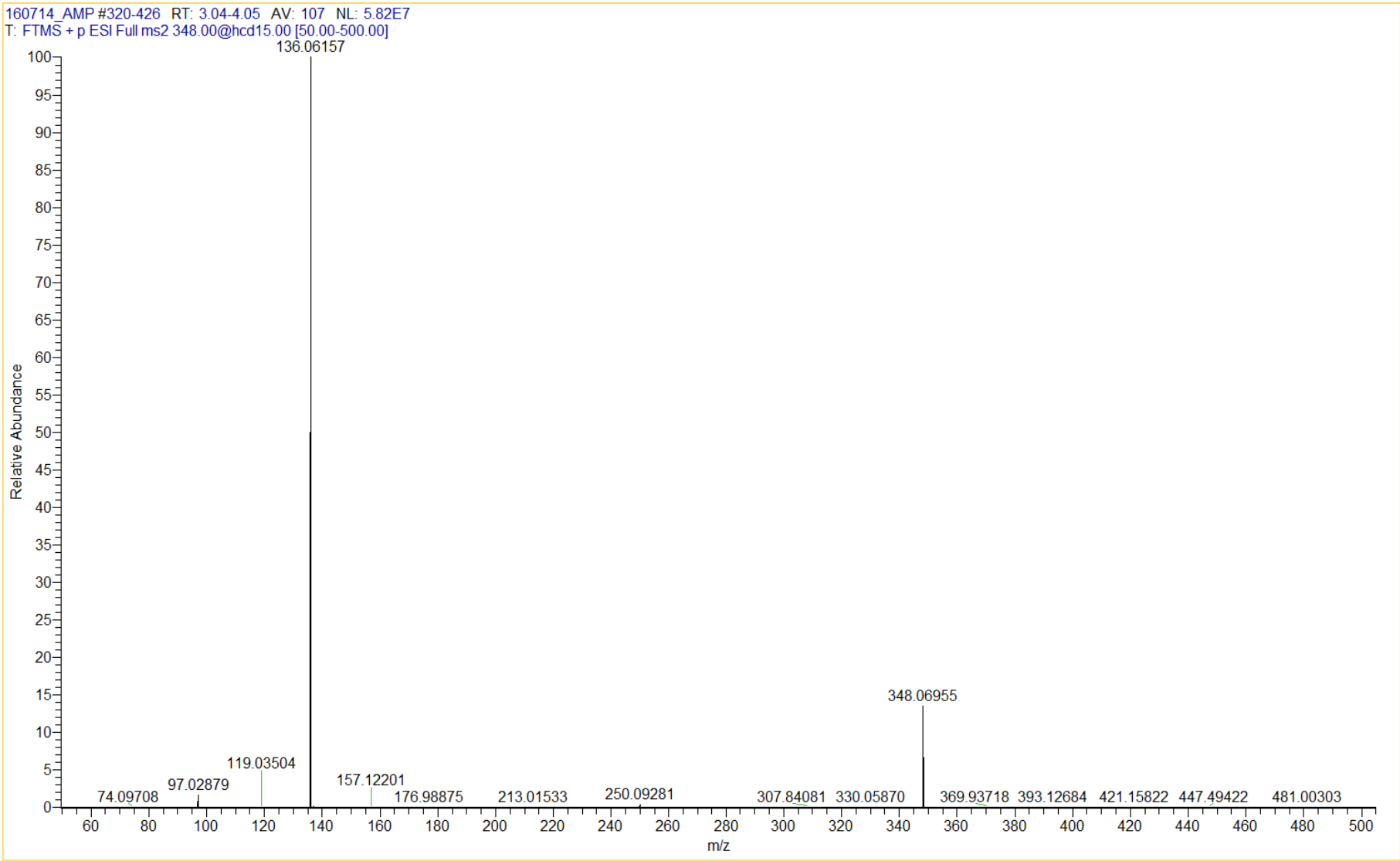
QTRAP 3200 AB Sciex, AMP, CE 45 ESI (-), EPI



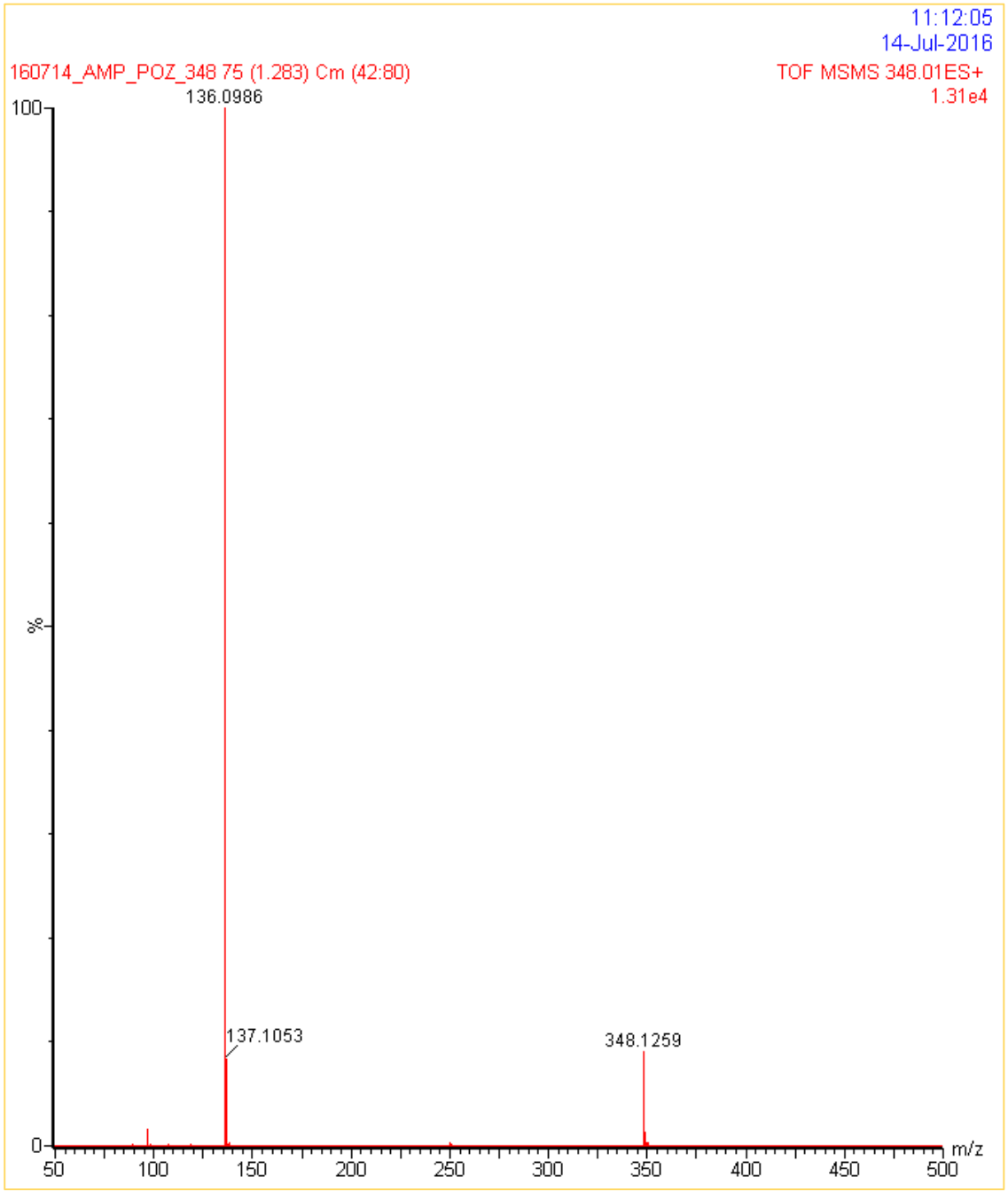
API 3200 AB Sciex, AMP, CE 40 ESI (-)



QExactive Thermo, AMP, CE 15 ESI (+)



QTOF Waters, AMP, CE 12 ESI (+)



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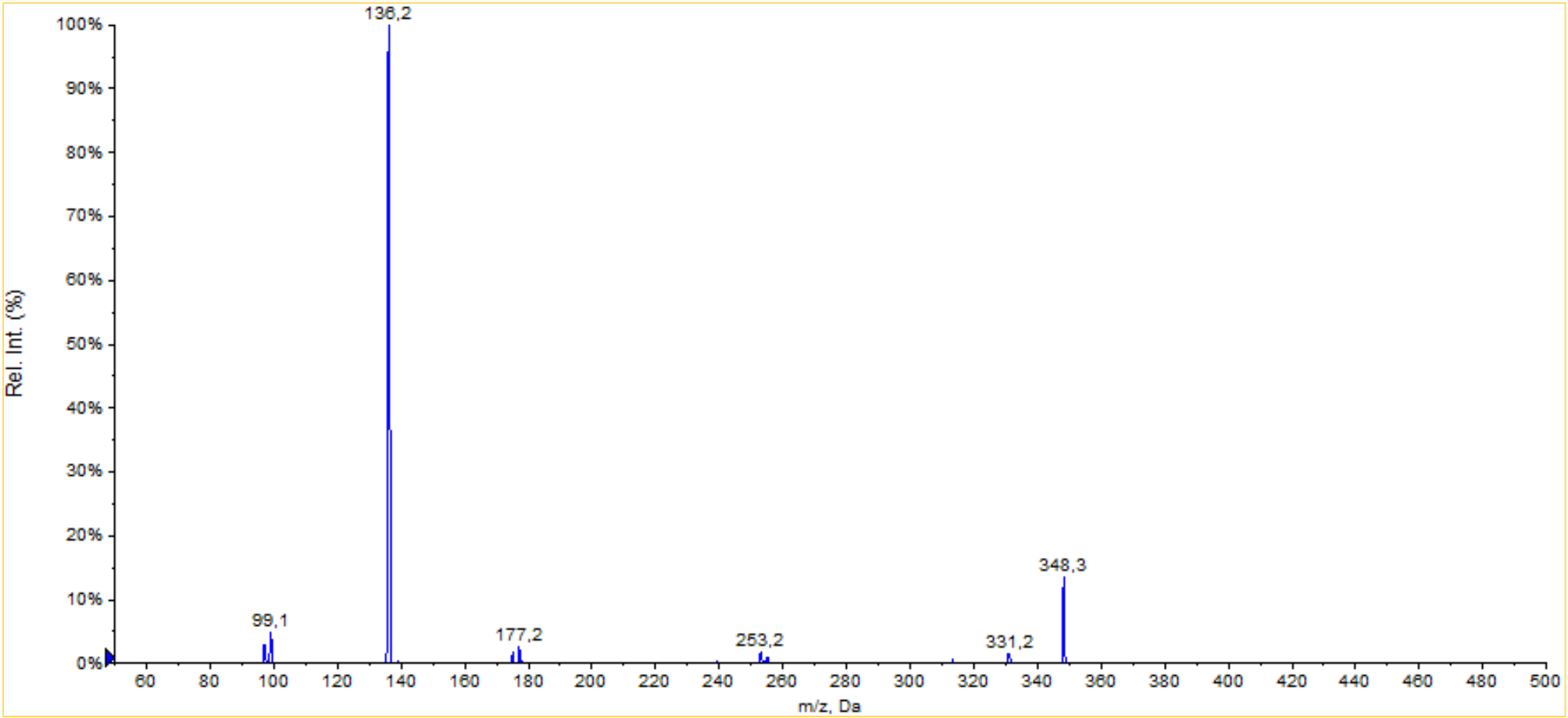

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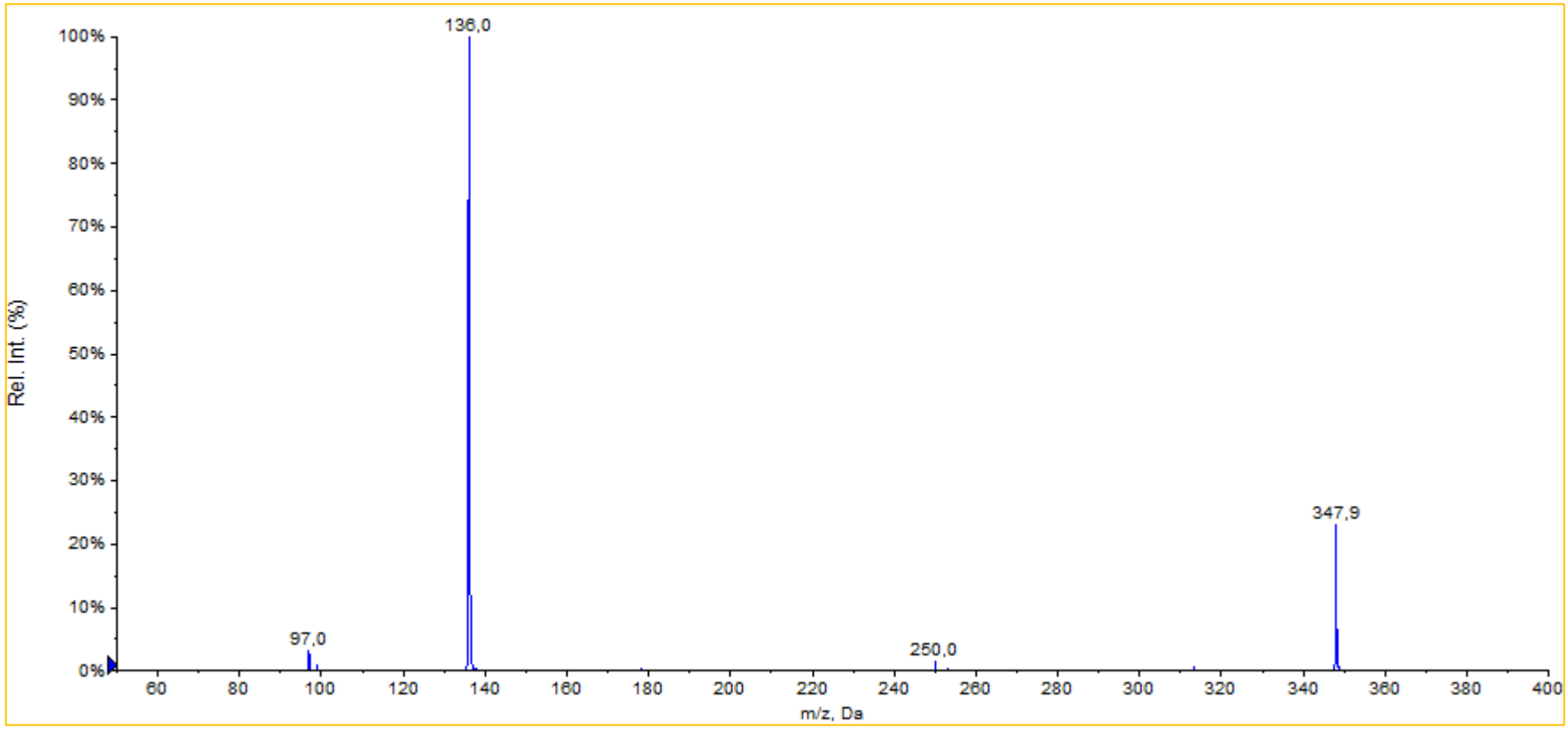
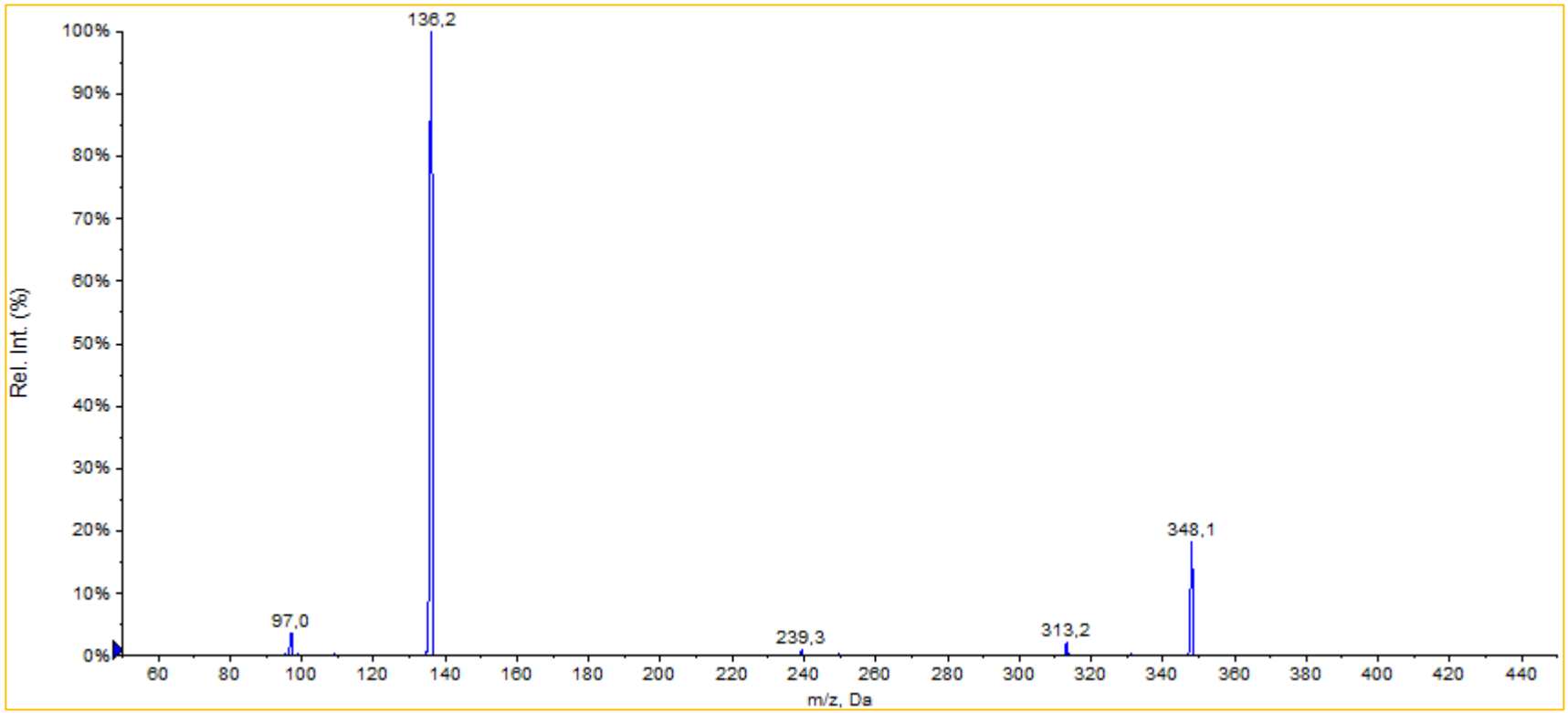


API 3200 AB Sciex, AMP, CE 25 ESI (+)



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