SUPPORTING INFORMATION

Topoisomerase Inhibitors Addressing Fluoroquinolone Resistance in Gram-negative Bacteria

Colin K. Skepper,[*,†] *Duncan Armstrong,*[§] *Carl J. Balibar,*[†] *Daniel Bauer,*[‡] *Cornelia Bellamacina,*[†] *Bret M. Benton,*[†] *Dirksen Bussiere,*[†] *Gianfranco De Pascale,*[†] *Javier De Vicente,*[†] *Charles R. Dean,*[†] *Bhavesh Dhumale,*[⊥] *L. Mark Fisher,*[¶] *John Fuller,*[†] *Mangesh Fulsunder,*[⊥] *Lauren M. Holder,*[†] *Cheng Hu,*[†] *Bhavin Kantariya,*[⊥] *Guillaume Lapointe,*[†] *Jennifer A. Leeds,*[†] *Xiaolin Li,*[†] *Peichao Lu,*[†] *Anatoli Lvov,*[§] *Sylvia Ma,*[†] *Shravanthi Madhavan,*[†] *Swapnil Malekar,*[†] *David McKenney,*[†] *Wosenu Mergo,*[†] *Louis Metzger,*[†] *Heinz E. Moser,*[†] *Daniel Mutnick,*[†] *Jonas Noeske,*[†] *Colin Osborne,*[†] *Ashish Patel,*[⊥] *Darshit Patel,*[⊥] *Tushar Patel,*[⊥] *Krunal Prajapati,*[⊥] *Katherine R. Prosen,*[†] *Folkert Reck,*[†] *Daryl L. Richie,*[†] *Alice Rico,*[†] *Mark R. Sanderson,*[∥] *Shailesh Satasia,*[⊥] *William S. Sawyer,*[†] *Jogitha Selvarajah,*[¶] *Nirav Shah,*[⊥] *Kartik Shanghavi,*[⊥] *Wei Shu,*[†] *Katherine V. Thompson,*[†] *Martin Traebert,*[‡] *Anand Vala,*[⊥] *Lakhan Vala,*[⊥] *Dennis A. Veselkov,*[∥] *Jason Vo,*[†] *Michael Wang,*[†] *Marcella Widya,*[†] *Sarah L. Williams,*[†] *Yongjin Xu,*[†] *Qin Yue,*[†] *Richard Zang,*[†] *Bo Zhou*[†] *and Alexey Rivkin*[†]

[†]Novartis Institutes for BioMedical Research, Emeryville, California 94608, United States

[‡]Novartis Institutes for BioMedical Research, 4002 Basel, Switzerland

[§]Novartis Institutes for BioMedical Research, Cambridge, Massachusetts 02139, United States

[⊥] Piramal Discovery Solutions, Pharmaceutical Special Economic Zone, Sarkhej Bavla Highway, Ahmedabad, Gujarat 382213, India

[¶] Molecular and Clinical Sciences Research Institute, St George's, University of London, London SW17 0RE, UK

[|] Randall Centre for Cell and Molecular Biophysics, King's College, Guy's Campus, London Bridge, London SE1 1UL, UK

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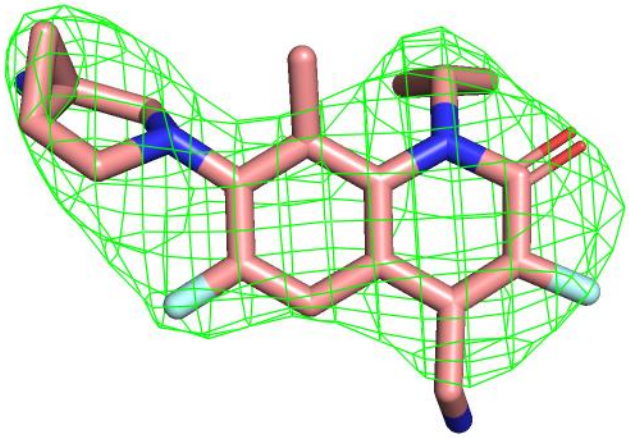
Supplementary Tables and Figures

Supplementary Table 1. Crystallographic data table for the structure of *K. pneumoniae* topoisomerase IV bound to compound **34**.

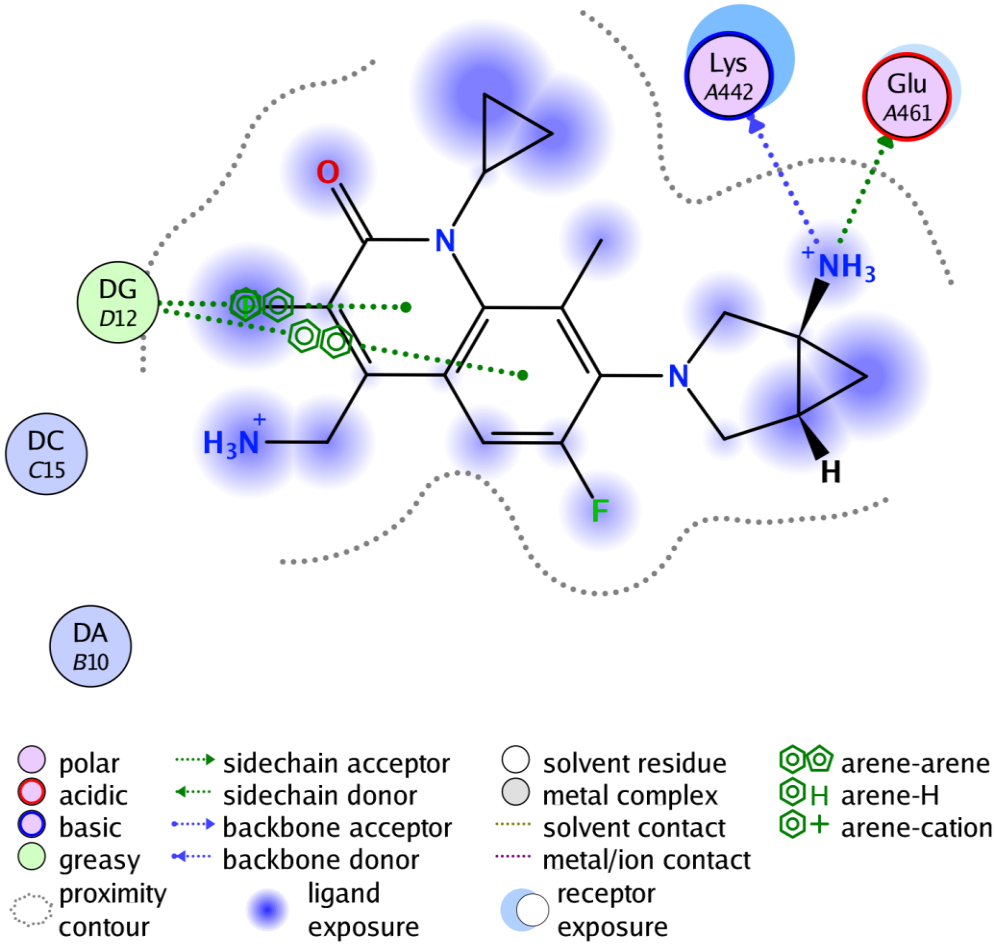
Data collection statistics	
Data set	Native
X-ray source	ALS BL5.0.2
Space group	$P2_1$ (two molecules per asymmetric unit)
Unit cell parameters	*a* = 94.590 Å, *b* = 157.481 Å, *c* = 144.060 Å, β = 94.930°
Wavelength (Å)	1.0000
Resolution (Å)	94.24-3.20 (3.37-3.20)
Observations	217597 (32804)
Unique reflections	69233 (10085)
Completeness (%)	99.8 (99.8)
Multiplicity	3.1 (3.3)
R-merge	0.257 (1.906)
I/σ (*I*)	4.7 (0.8)
CC(1/2)	0.971 (0.195)
Refinement statistics	
Resolution range (Å)	82.08-3.20 (3.314-3.20)
Reflections used in refinement	68999 (6743)
Reflections used for R-free	3262 (327)
R-work (%)	27.49 (38.43)
R-free (%)	30.12 (42.03)
Number of non-hydrogen atoms	21491
macromolecules	21316
ligands	165
solvent	10
Ramachandran favored/allowed/outliers (%)	97.17/2.75/0.08
RMS (bonds) (Å)	0.002
RMS (angles) (°)	0.48
Average B-factor (Å2)	92.01
macromolecules	92.07
ligands	86.57
solvent	63.9
PDB ID	6WAA

Data for the last resolution shell are in parentheses.

Supplementary Figure 1. Chemical structure of **34** and unbiased electron-density difference map (F_{obs}-F_{calc}) around the compound contoured at 3.0σ.

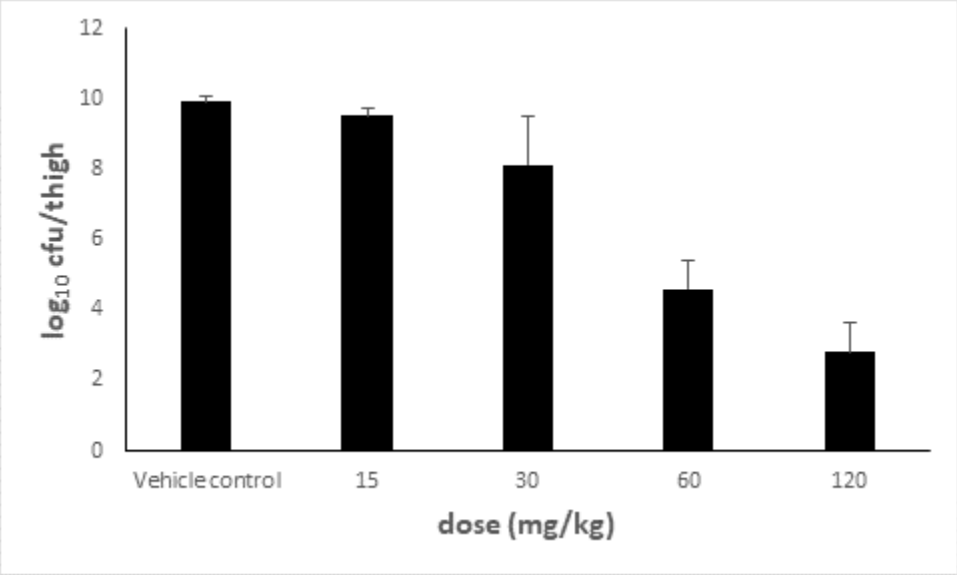


Supplementary Figure 2. Interaction diagram of compound **34** bound to topoisomerase IV from *K. pneumoniae*.

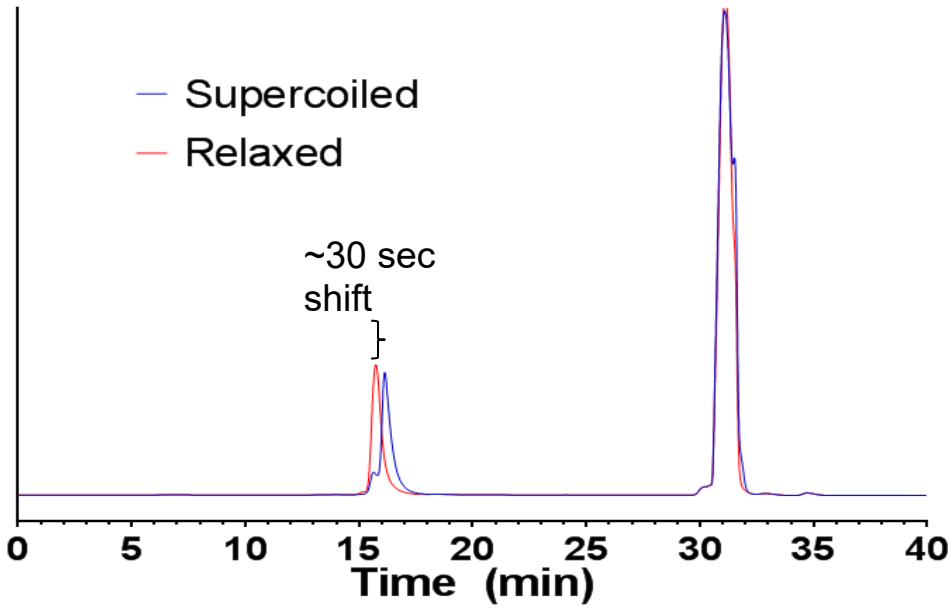


The interaction diagram was drawn with Molecular Operating Environment (MOE): *Molecular Operating Environment (MOE)*, 2019.01; Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2019**

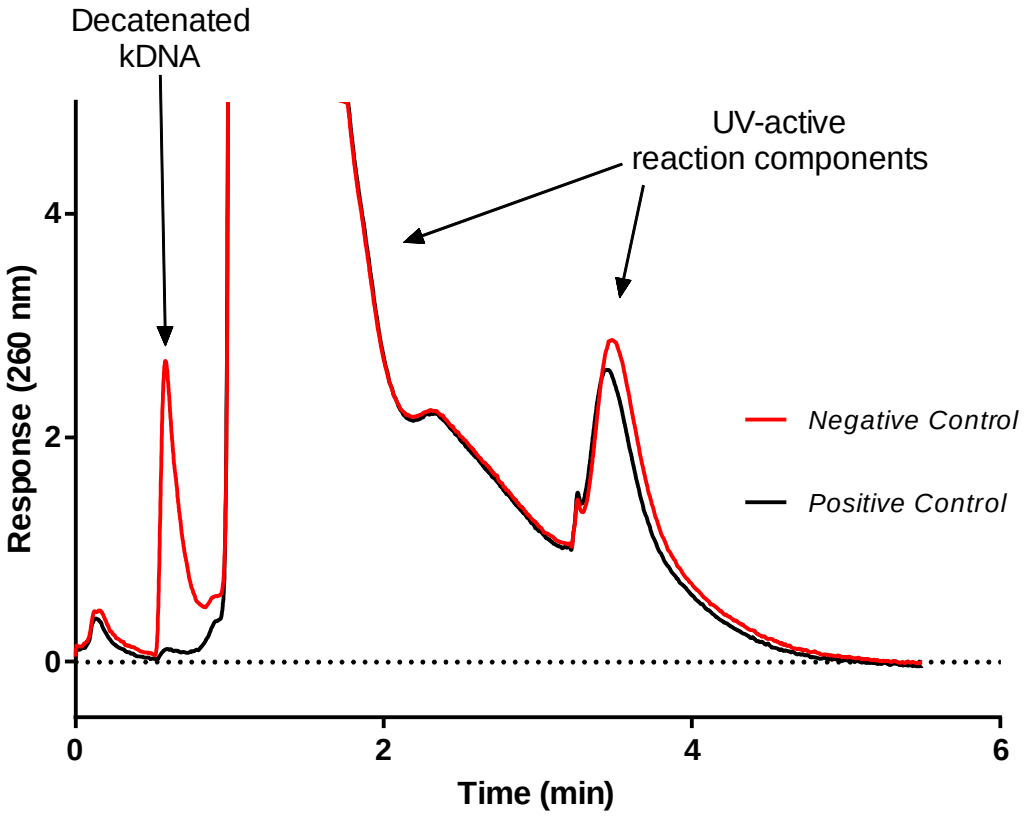
Supplementary Figure 3. CFU/Thigh determined for each dose of compound **34** in the neutropenic murine thigh infection model against *E. coli* ATCC 25922 (4 animals per dose group).



Supplementary Figure 4. *E. coli* gyrase DNA supercoiling assay: example chromatogram showing separation of supercoiled and relaxed DNA by size exclusion chromatography.

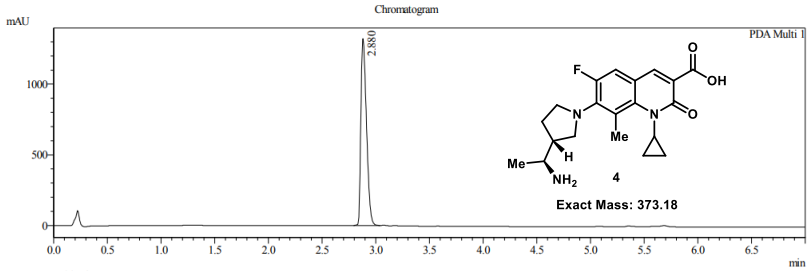


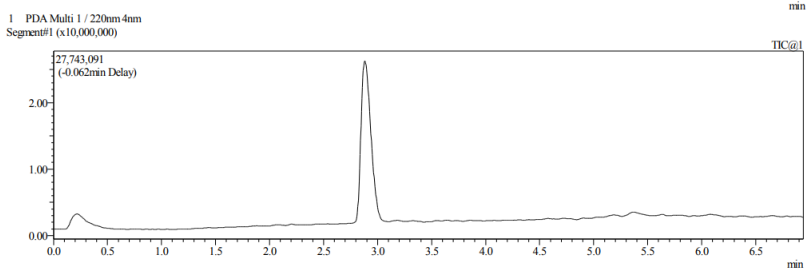
Supplementary Figure 5. *E. coli* Topoisomerase IV DNA decatenation assay: example chromatogram showing detection of decatenated DNA by size exclusion chromatography. Ciprofloxacin and gepotidacin were typically used as positive controls.

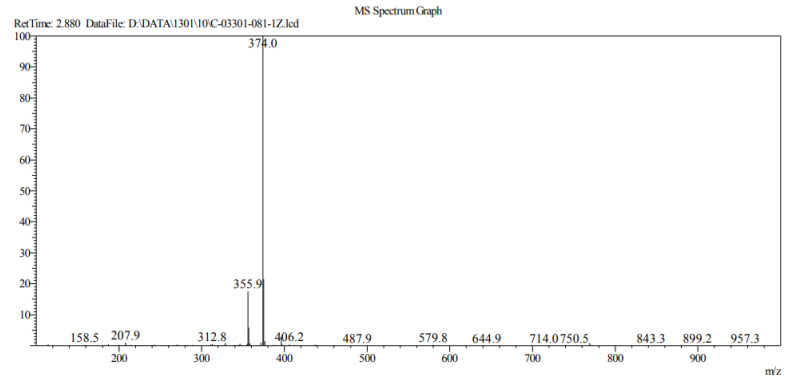


LC/MS Chromatograms and MS Data for Final Compounds

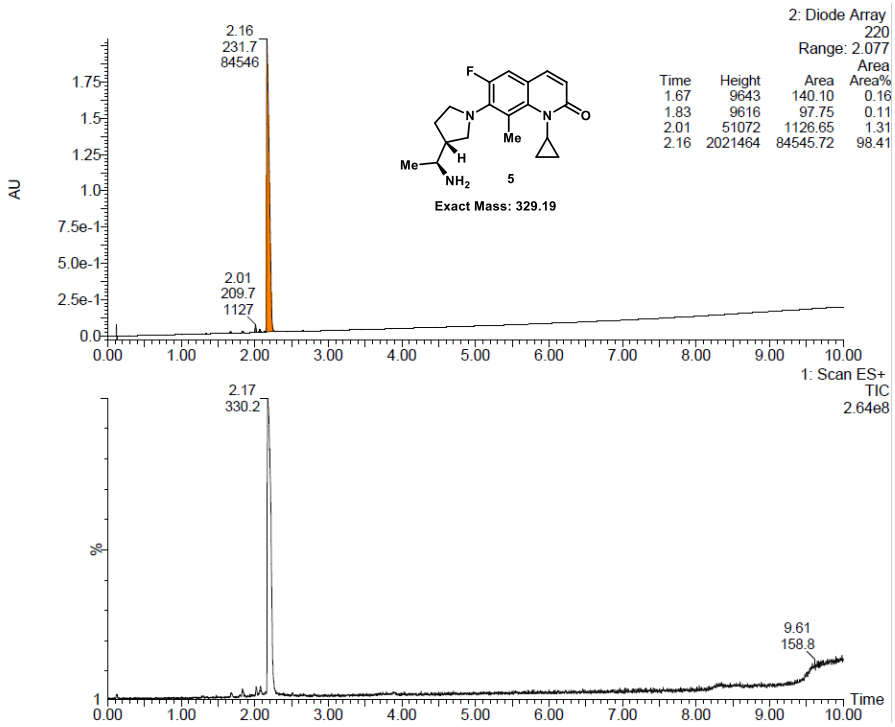
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid hydrochloride (4)



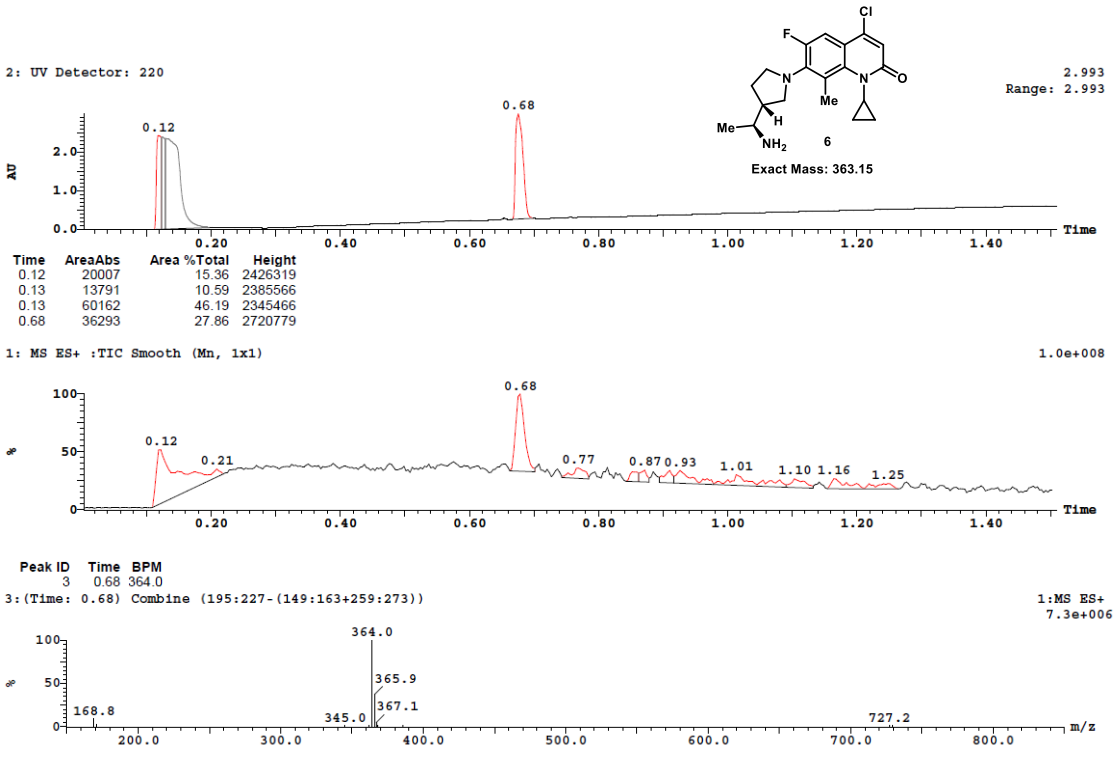




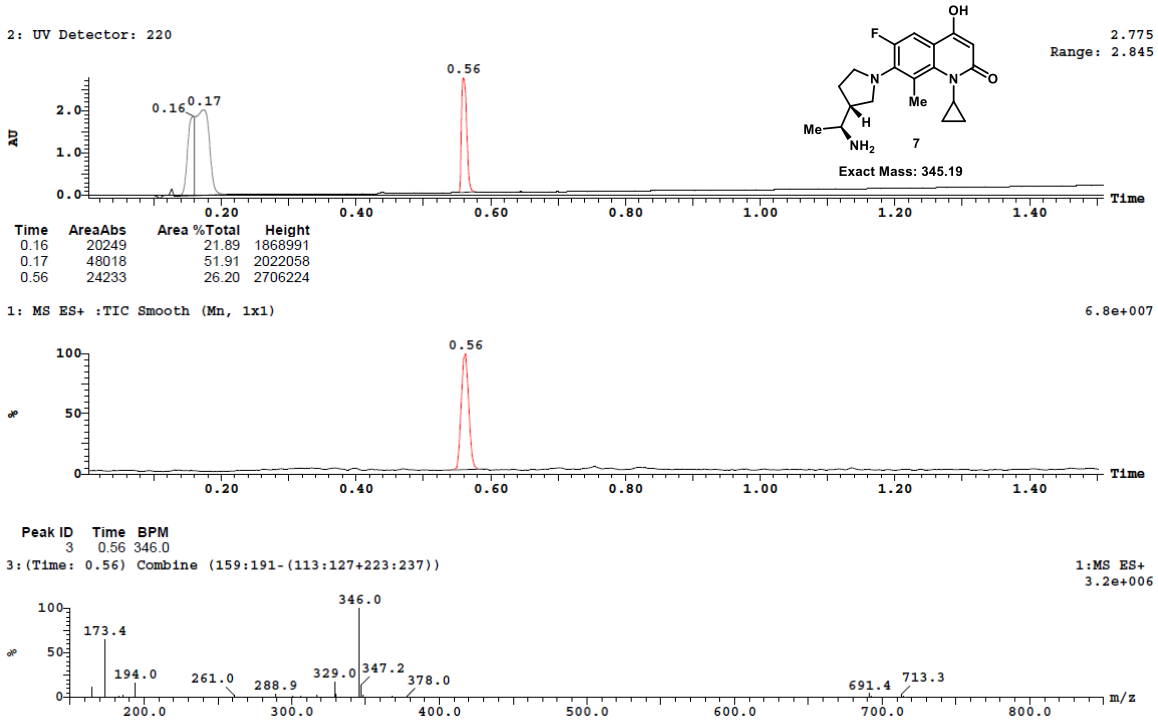
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one hydrochloride (5)



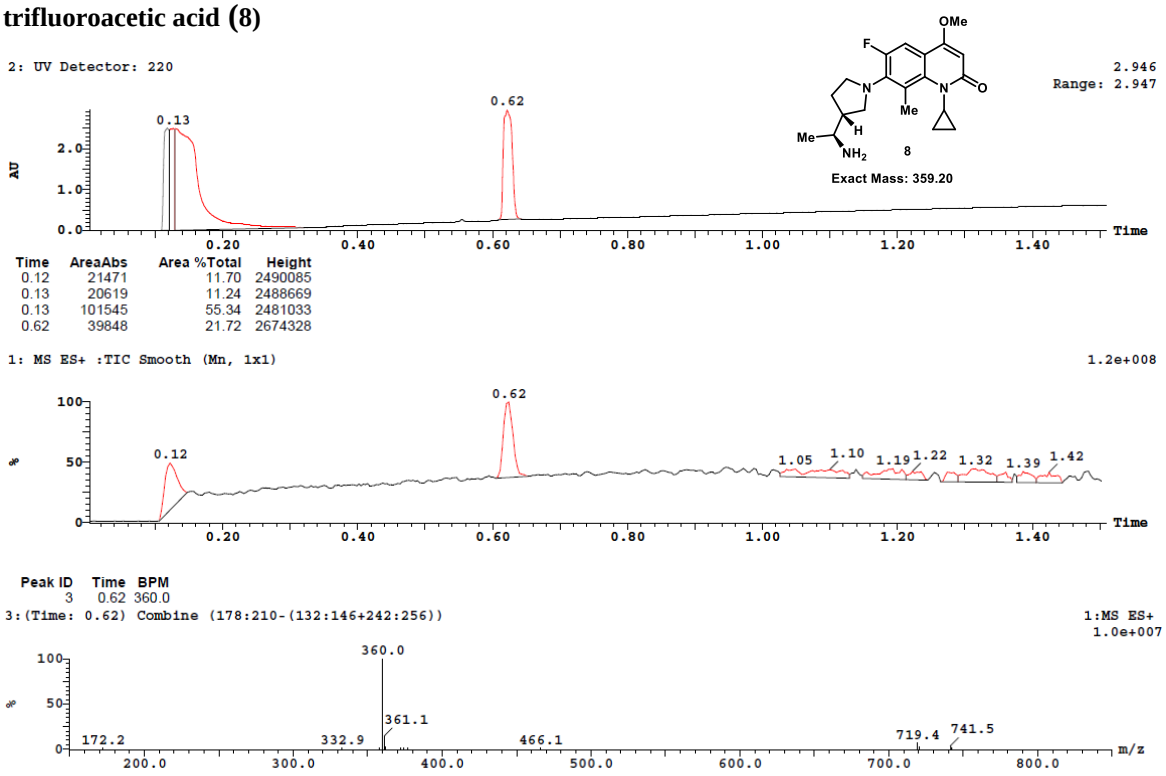
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-4-chloro-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (6)



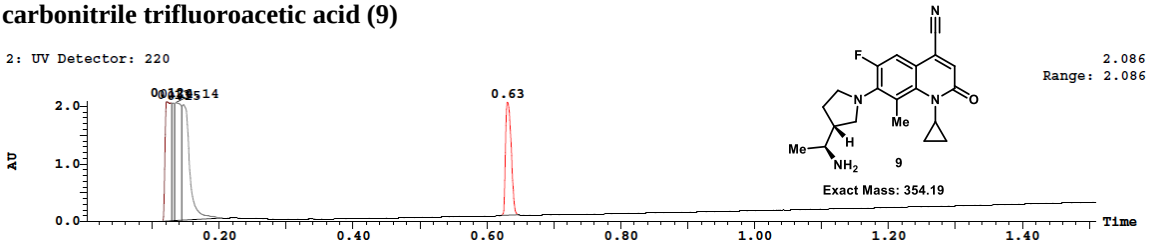
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-hydroxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (7)



7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-methoxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (8)



7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile trifluoroacetic acid (9)



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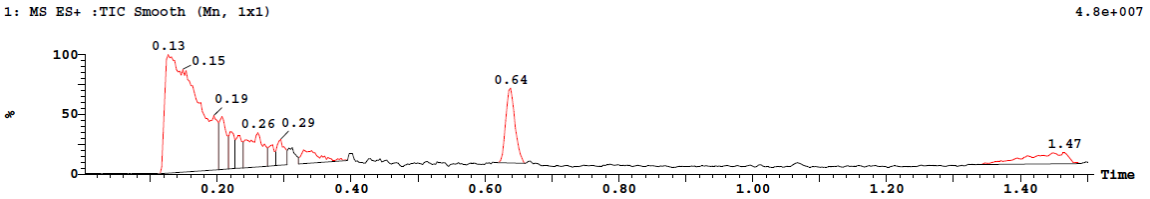
0.63	19202	19.51	1967151



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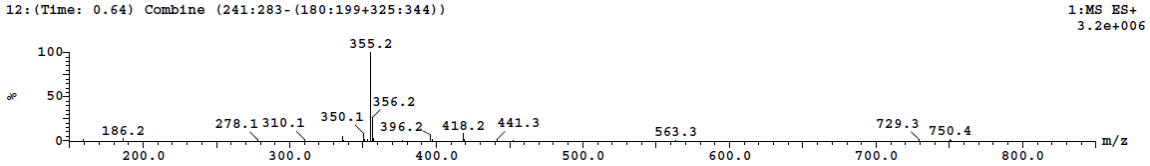
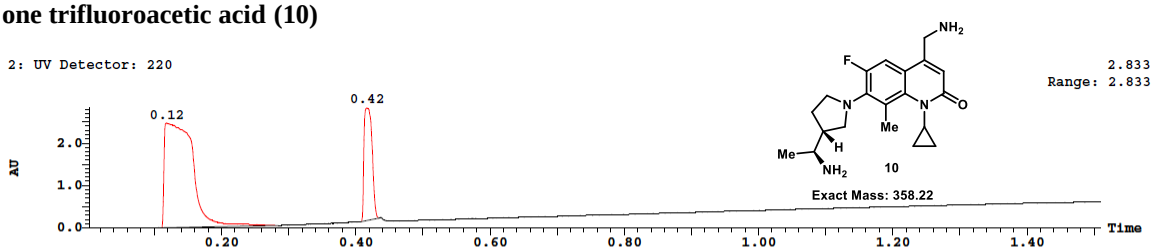


7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (10)



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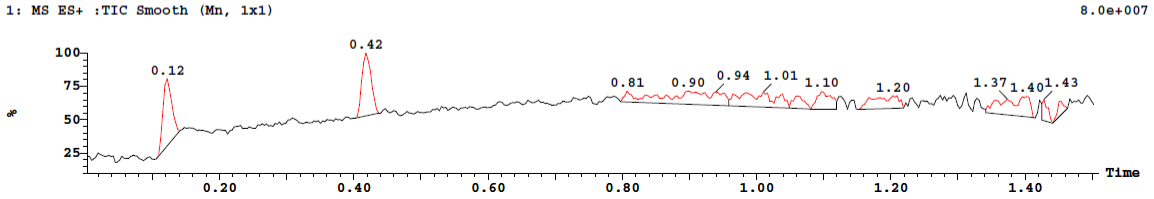

Peak ID Time BPM
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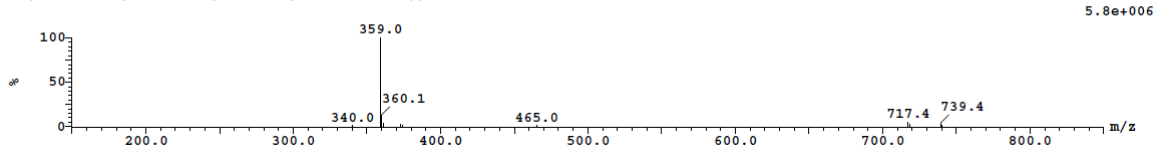
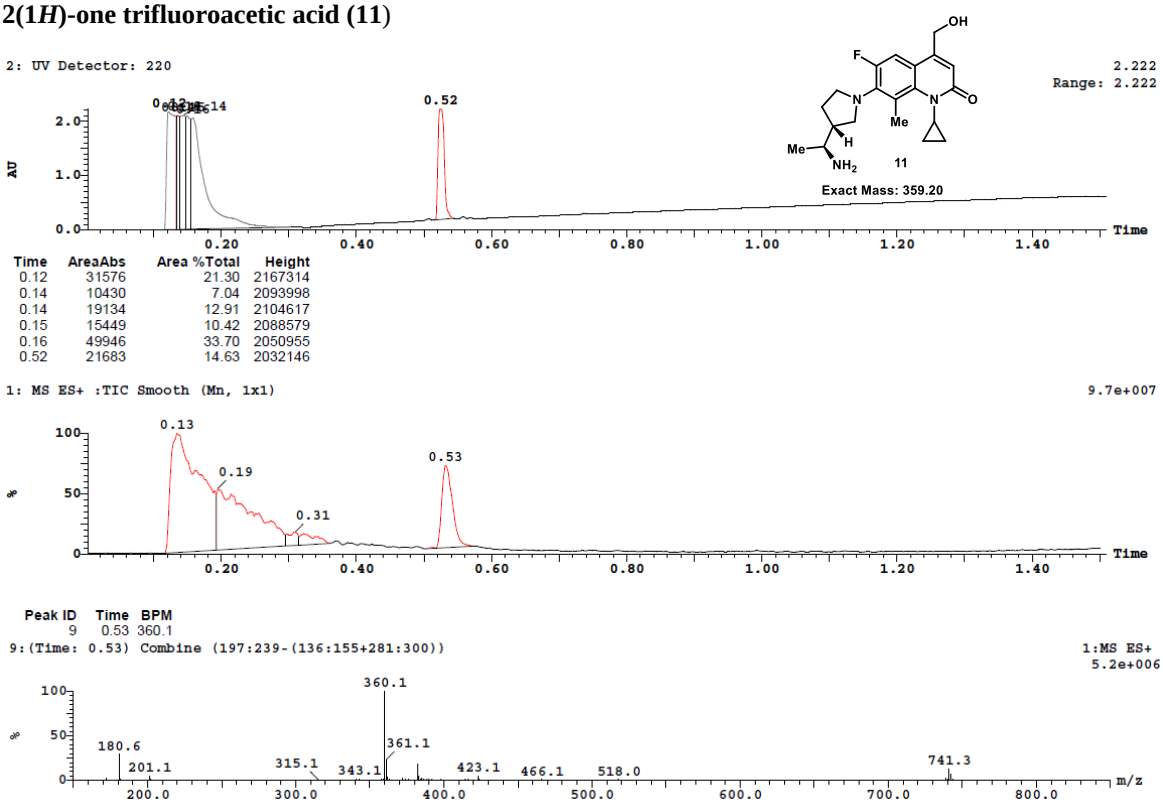
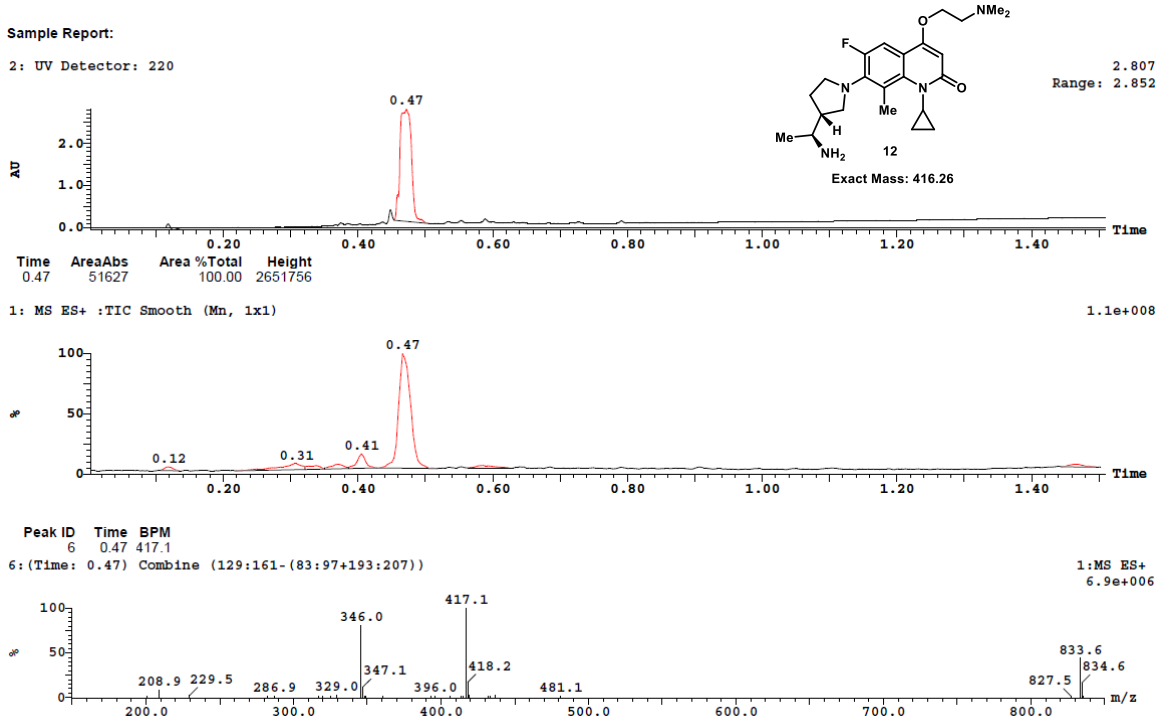


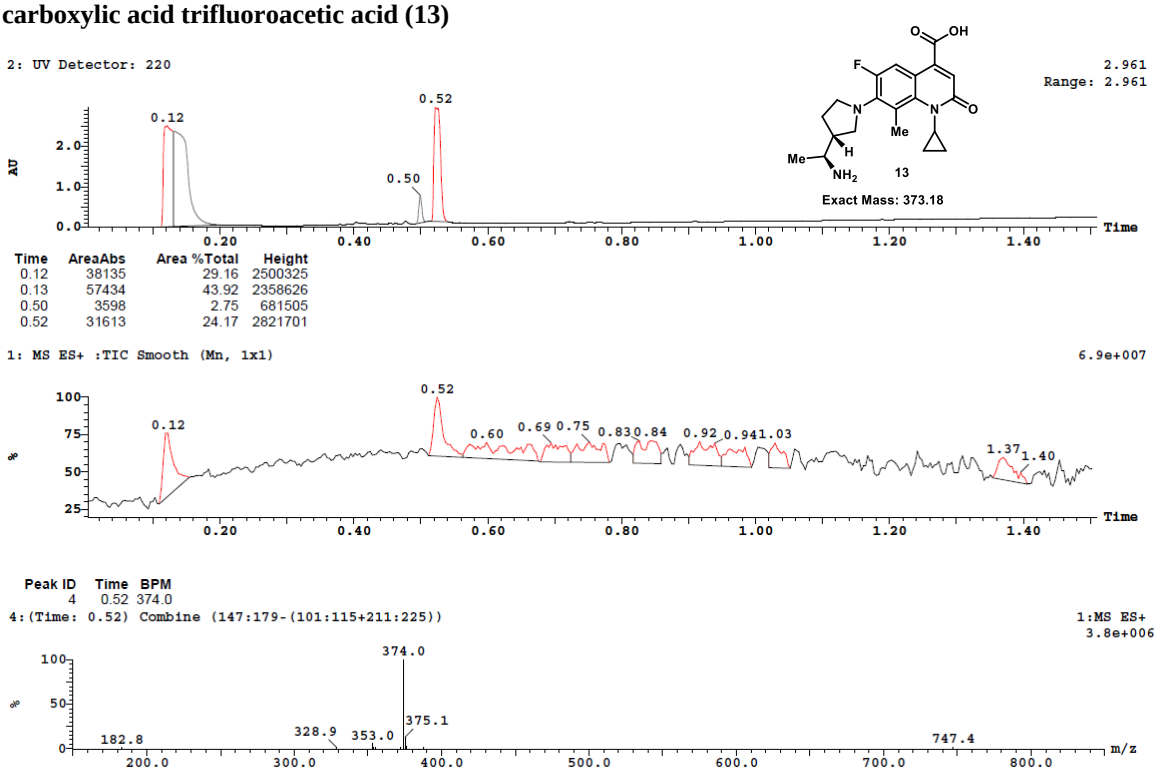
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-(hydroxymethyl)-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (11)



7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-4-(2-(dimethylamino)ethoxy)-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (12)



7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carboxylic acid trifluoroacetic acid (13)



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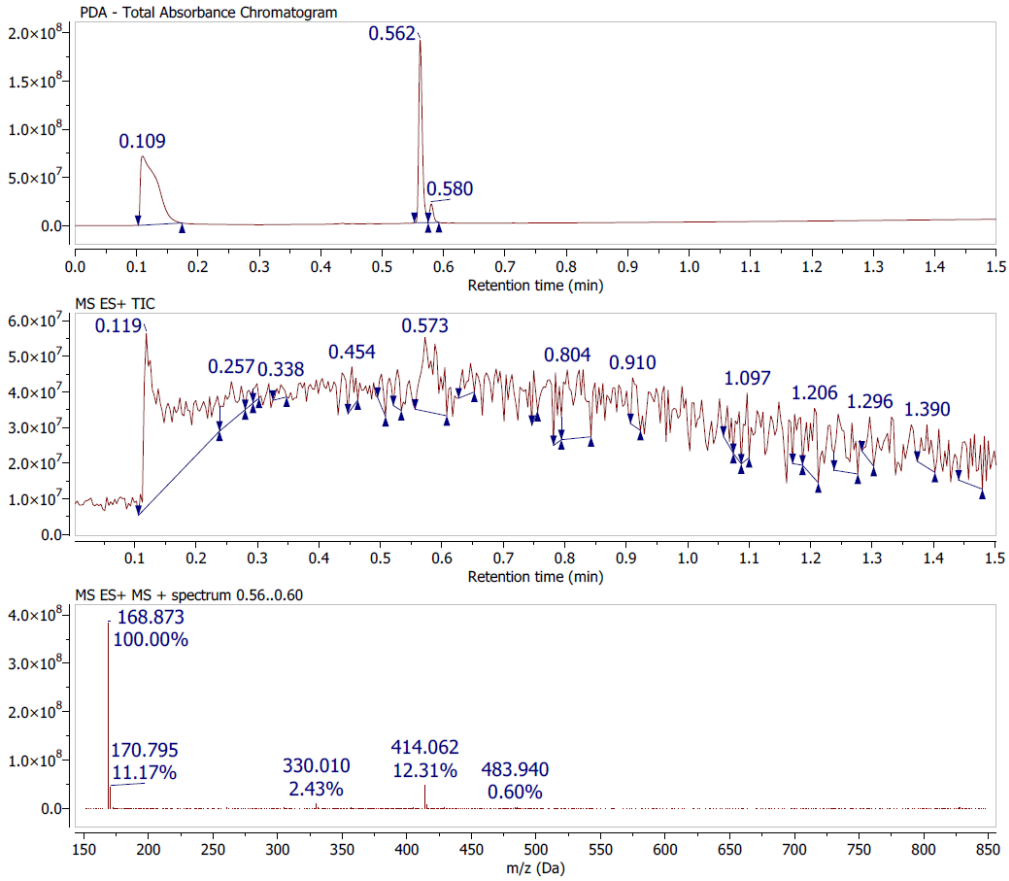
0.50	3598	2.75	681505
0.52	31613	24.17	2821701

7-((*R*)-3-((*S*)-1-aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-(5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)quinolin-2(1*H*)-one trifluoroacetic acid (14)



PDA (Area% >= 0.5%)

RT	Area %
0.11	61.11
0.56	35.02
0.58	3.87

TIC (Area% >= 0.5%)

RT	Area %	MS+
0.12	37.54	168.88
0.26	4.19	168.88
0.29	0.68	168.89
0.30	0.53	168.91
0.34	0.71	168.85
0.45	1.50	168.87
0.50	1.05	168.89
0.53	1.33	168.86
0.57	9.37	168.88
0.65	1.74	168.86
0.75	1.05	168.91
0.78	2.31	168.88
0.80	10.07	168.87
0.91	2.08	168.88
1.06	1.28	168.88
1.08	1.69	168.88
1.10	1.95	168.86
1.17	2.52	168.89
1.21	4.50	168.86
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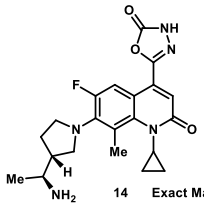
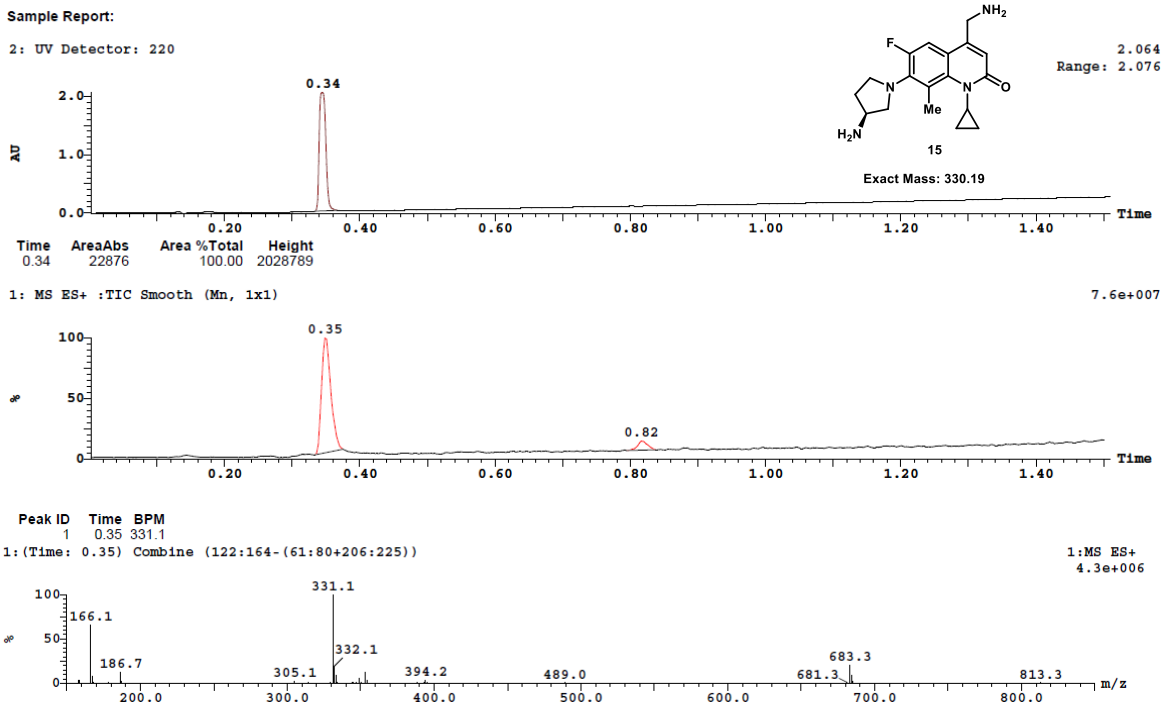
1.30	1.52	168.86
1.39	2.41	168.88
1.47	4.74	168.88

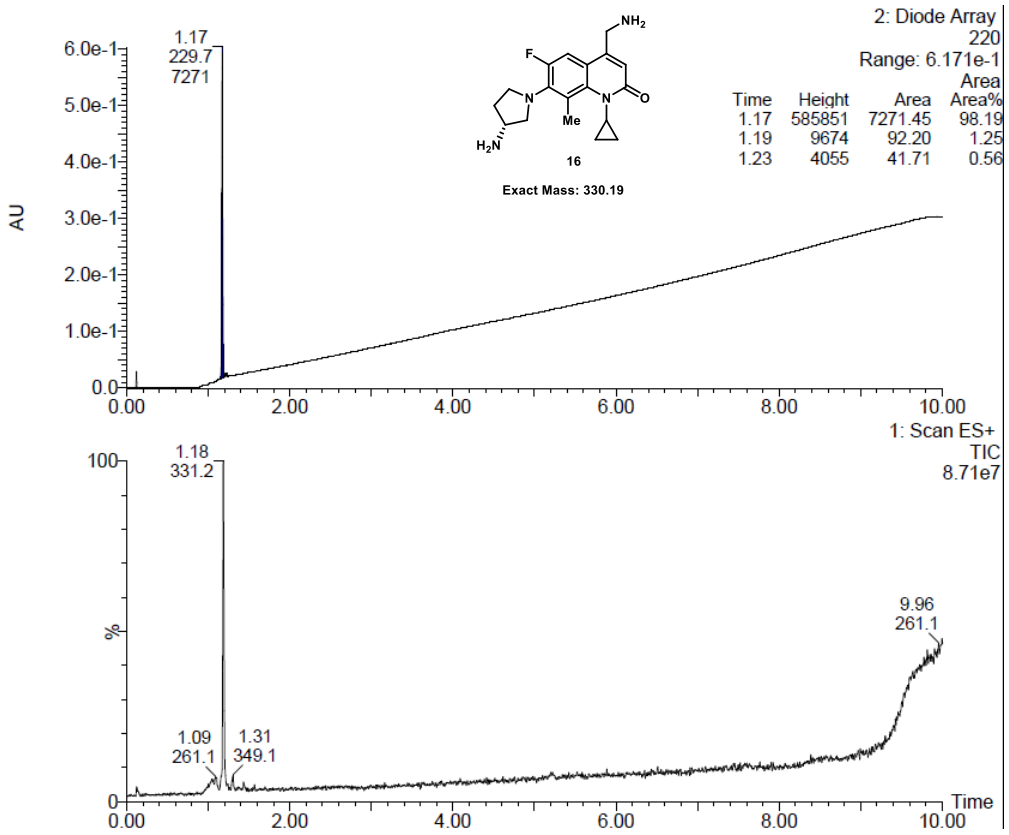


14 **Exact Mass: 413.19**

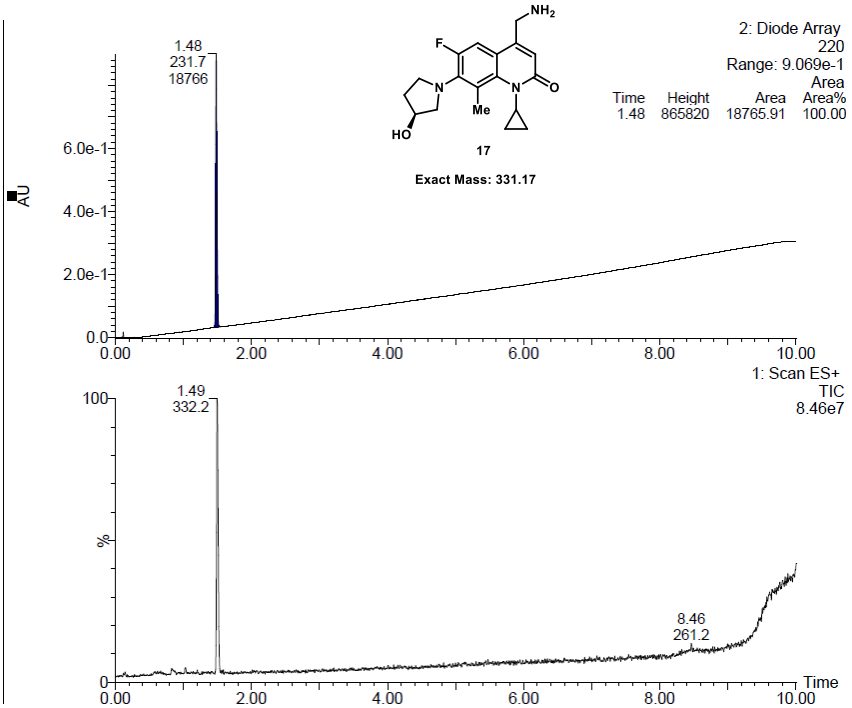
(*S*)-4-(Aminomethyl)-7-(3-aminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one hydrochloride (15)



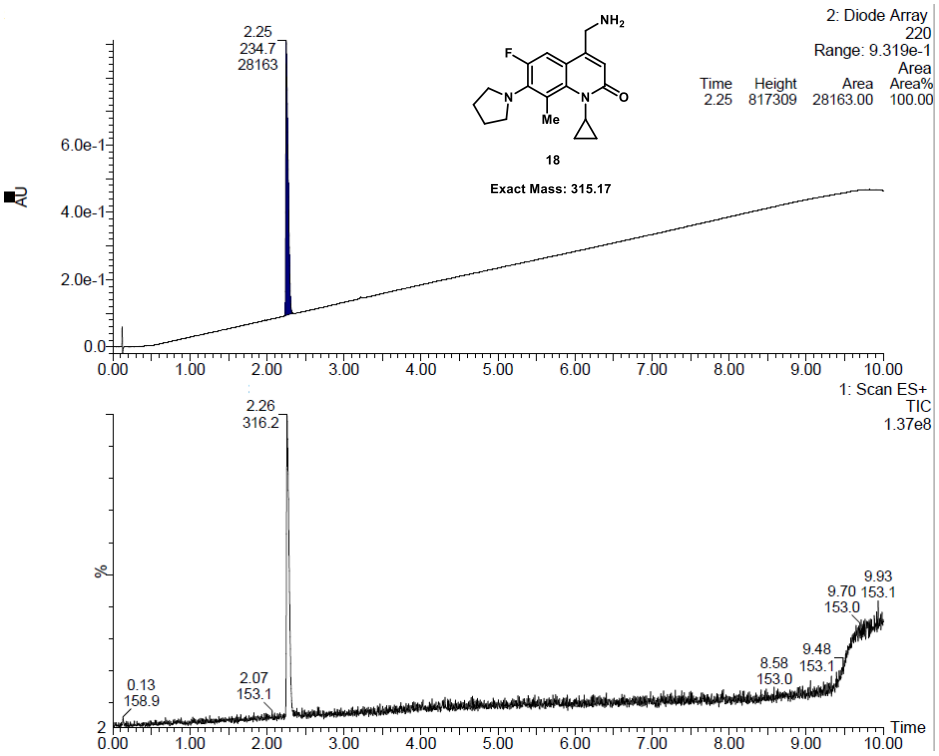
(*R*)-4-(Aminomethyl)-7-(3-aminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (16)



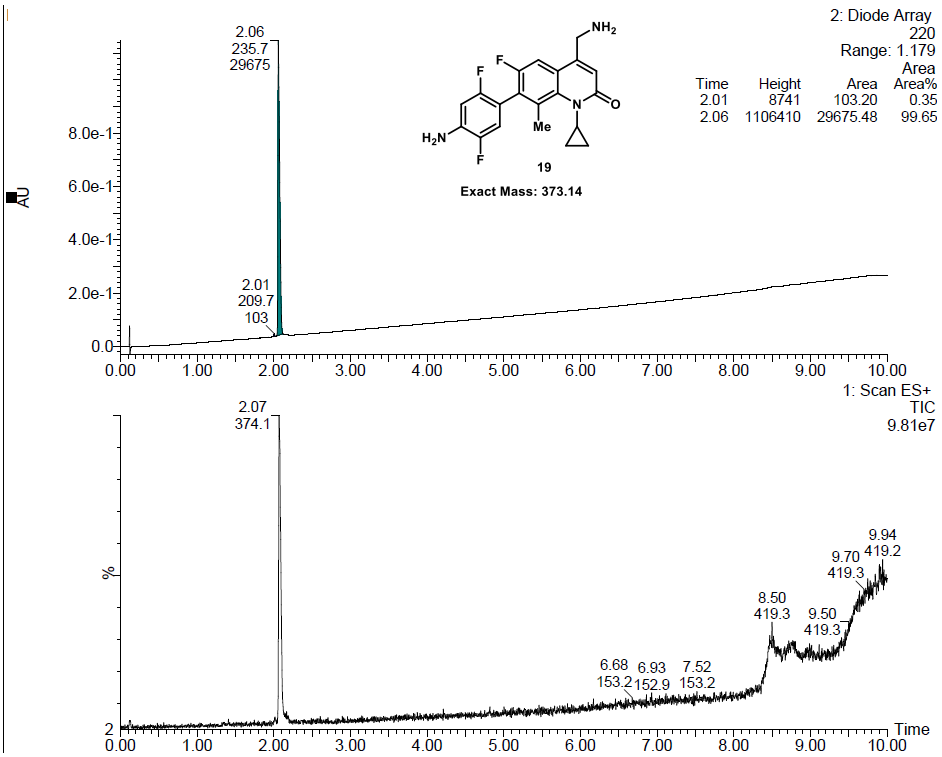
(*S*)-4-(Aminomethyl)-1-cyclopropyl-6-fluoro-7-(3-hydroxypyrrolidin-1-yl)-8-methylquinolin-2(1*H*)-one hydrochloride (17)



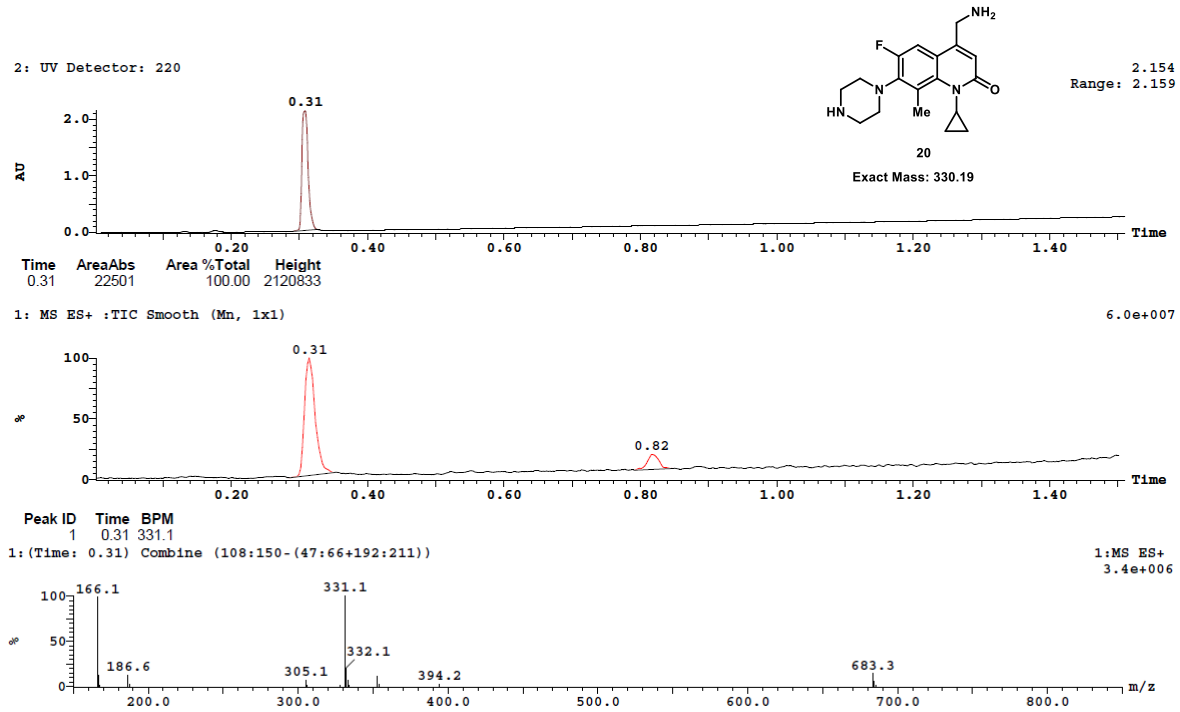
4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methyl-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (18)



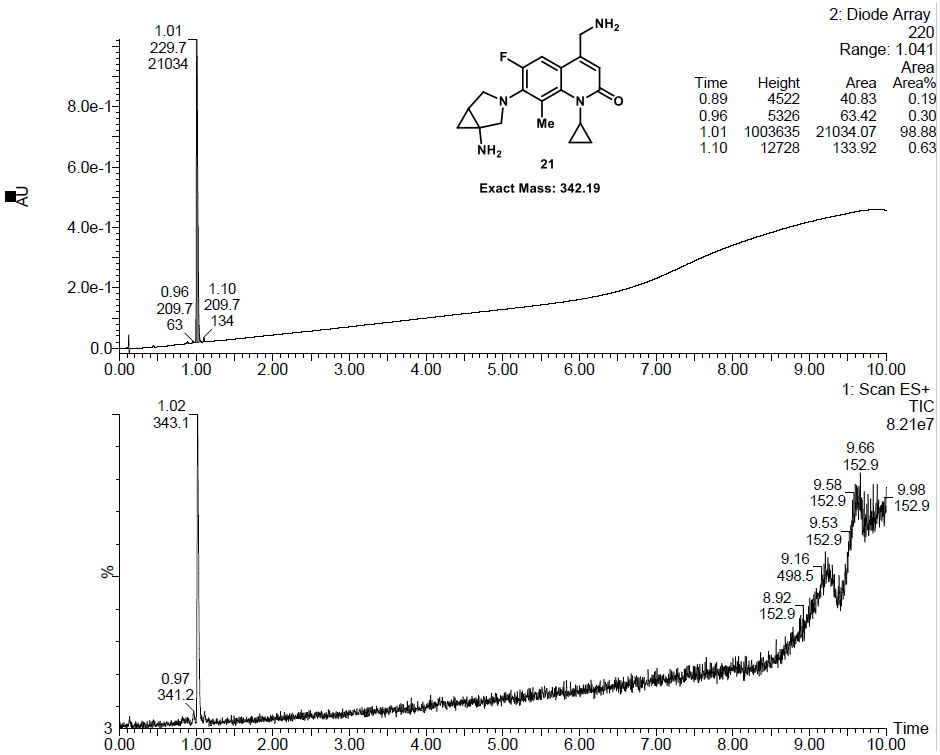
7-(4-Amino-2,5-difluorophenyl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (19)



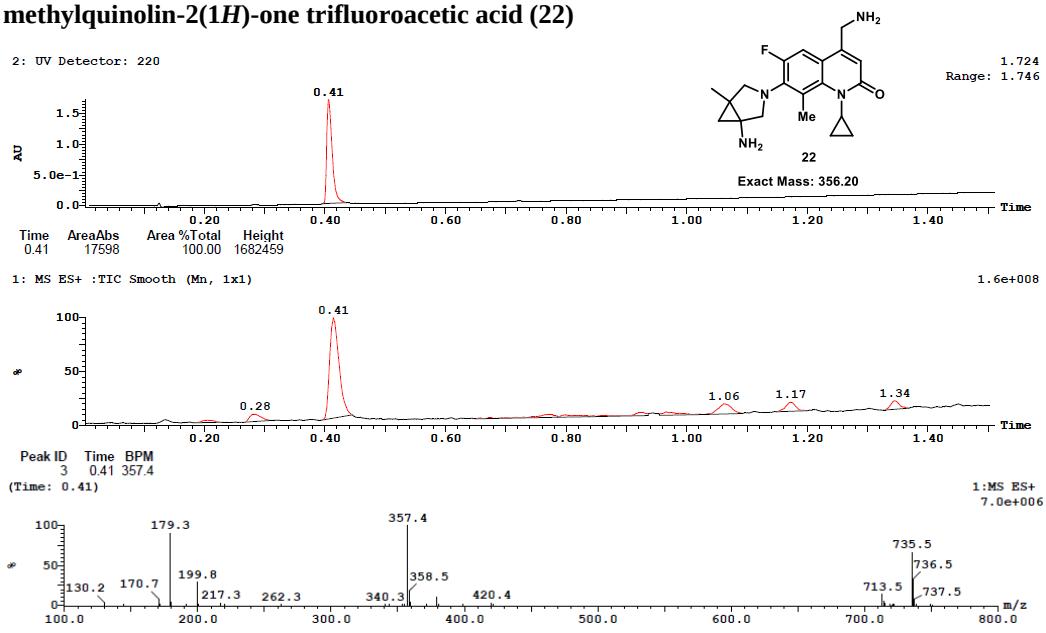
4-(Aminomethyl)-1-cyclopropyl-6-fluoro-8-methyl-7-(piperazin-1-yl)quinolin-2(1*H*)-one trifluoroacetic acid (20)



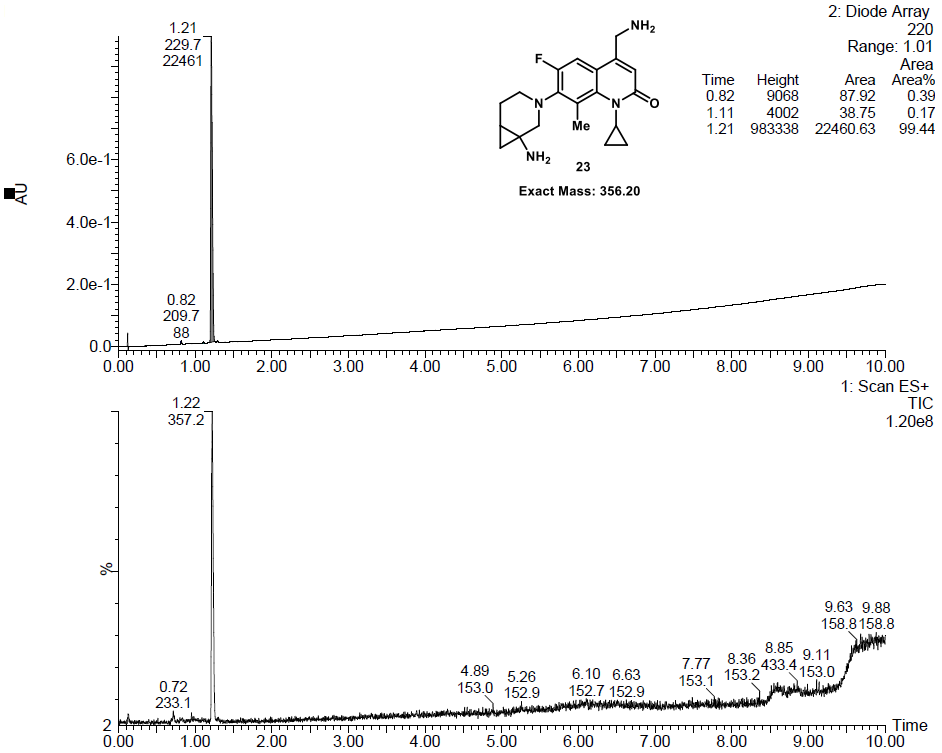
7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (21)



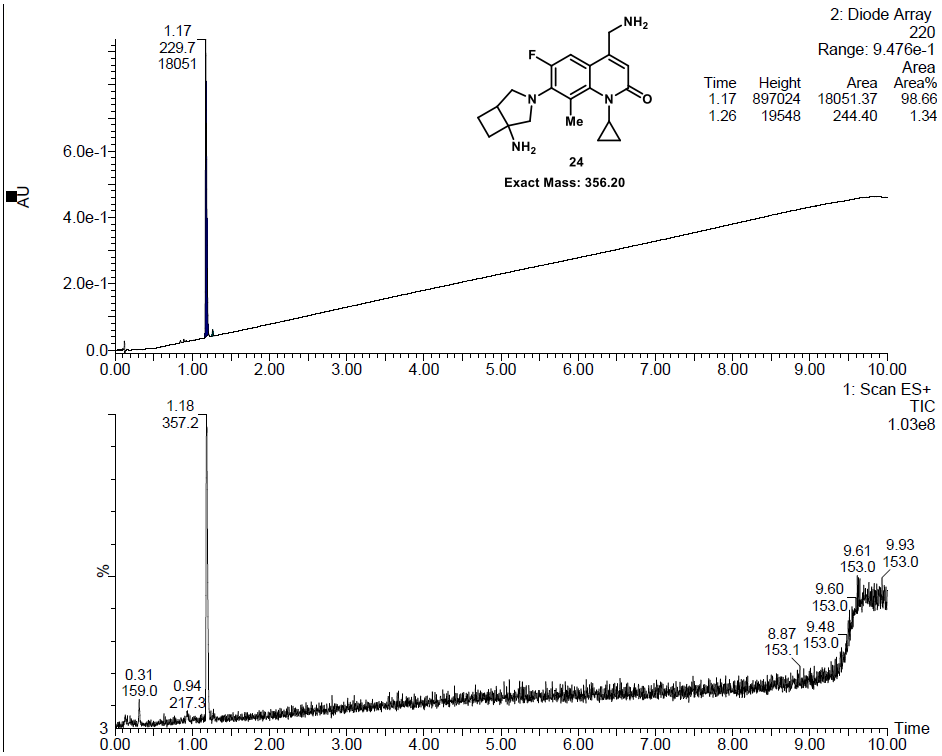
***rac*-7-((1*S*,5*R*)-1-Amino-5-methyl-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (22)**



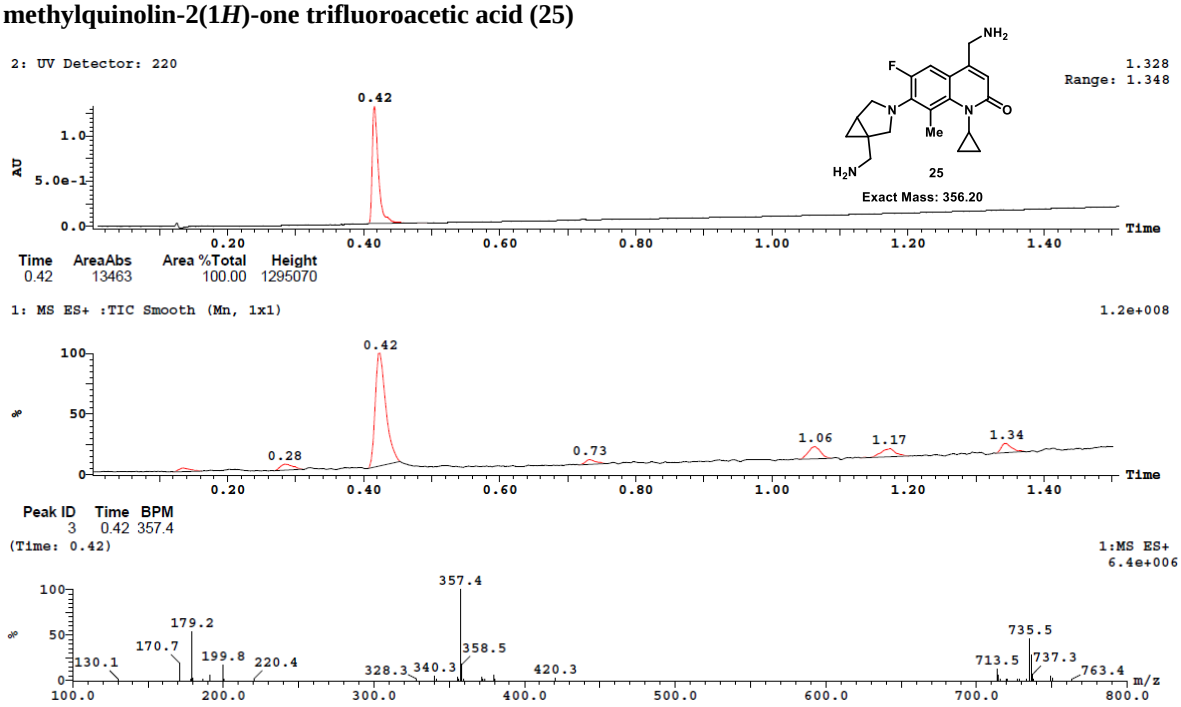
7-(1-amino-3-azabicyclo[4.1.0]heptan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one (23)



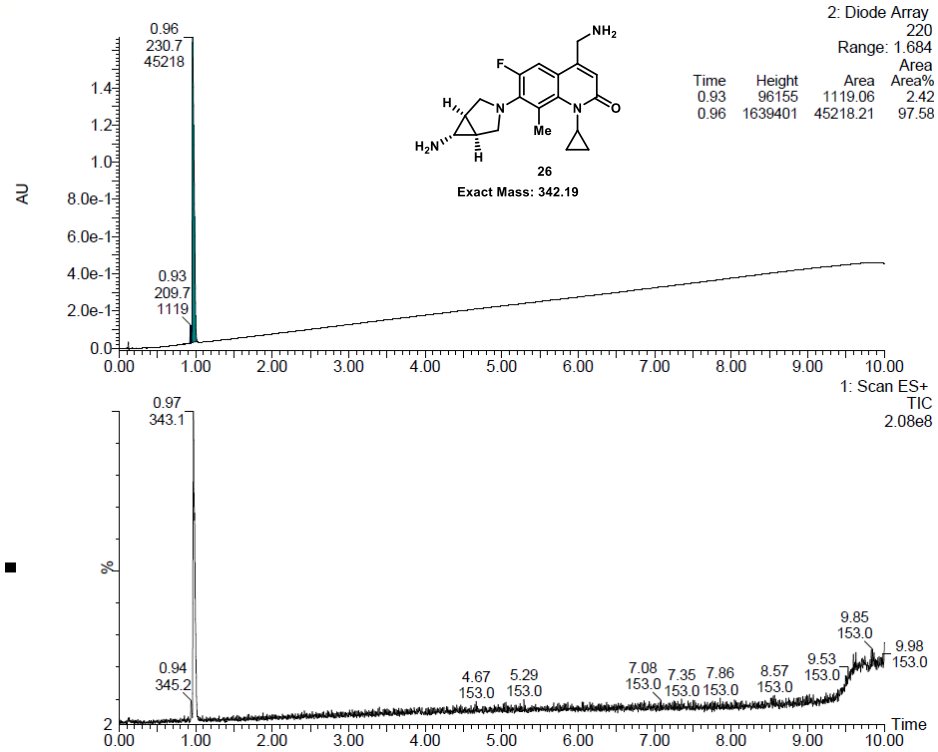
***rac*-7-((1*S*,5*R*)-1-Amino-3-azabicyclo[3.2.0]heptan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (24)**



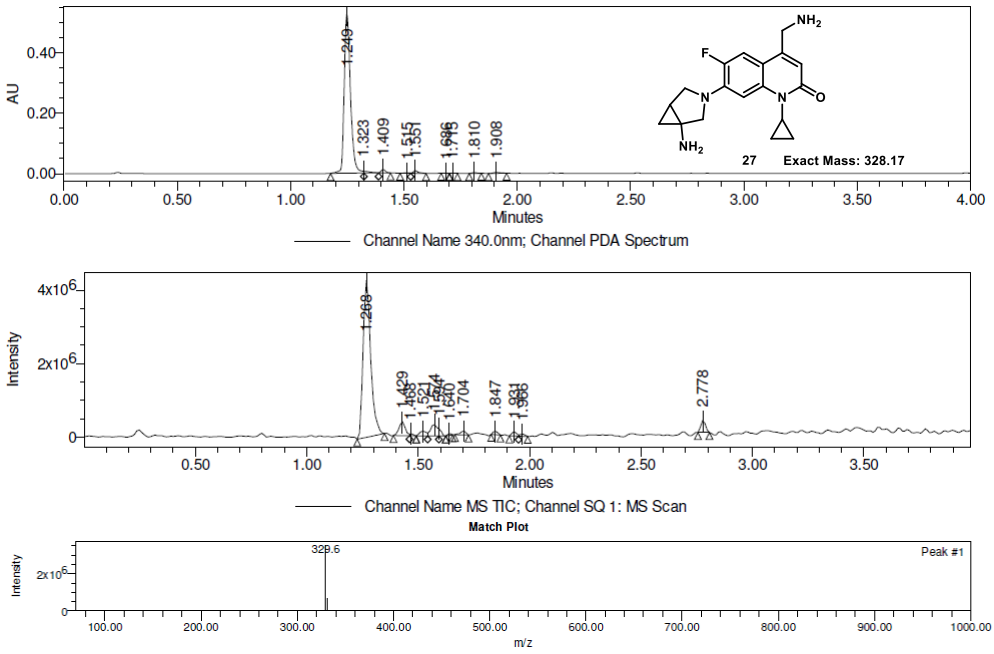
***rac*-4-(Aminomethyl)-7-(1-(aminomethyl)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (25)**



***rac*-7-((1*R*,5*S*,6*s*)-6-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (26)**



7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoroquinolin-2(1*H*)-one hydrochloride (27)

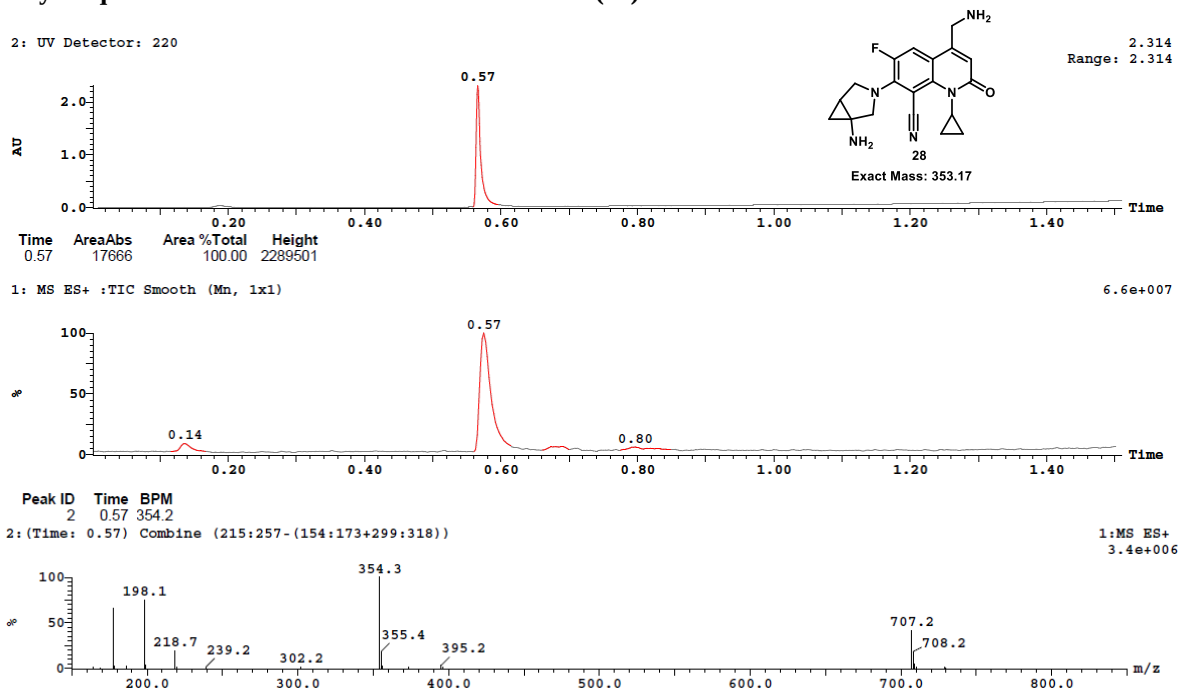


Base Peak 329.58 Channel Description 1: 70.00-1000.00 ES+, Centroid, CV=30 - AVG (0.0:1.2;1.9:3.9) x 30.000 Th: 0.100 - AVG (0.0:1.2;1.9:3.9) x 30.000 Th: 0.100 Retention Time 1.268

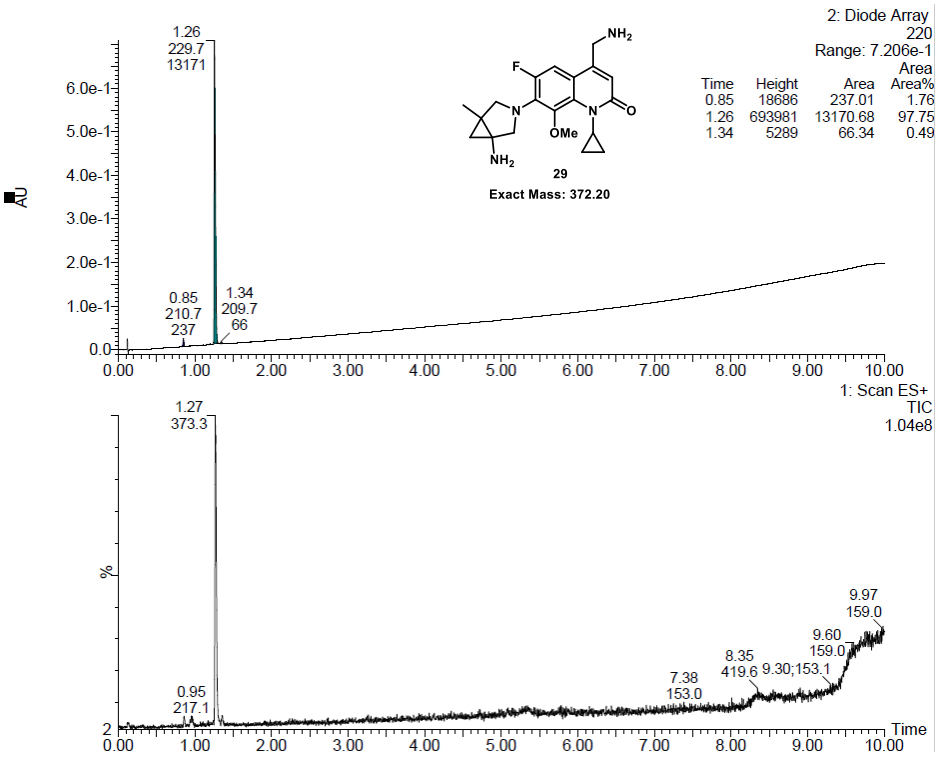
Peak Results
Channel: PDA Spectrum

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.249		524342	1005329	95.02	PDA Spectrum	340.0nm
2	1.323		6339	13615	1.29	PDA Spectrum	340.0nm
3	1.409		11260	15400	1.46	PDA Spectrum	340.0nm
4	1.515		1205	1681	0.16	PDA Spectrum	340.0nm
5	1.551		8549	12516	1.18	PDA Spectrum	340.0nm
6	1.686		292	256	0.02	PDA Spectrum	340.0nm
7	1.715		474	522	0.05	PDA Spectrum	340.0nm
8	1.810		2385	3096	0.29	PDA Spectrum	340.0nm
9	1.908		3202	5619	0.53	PDA Spectrum	340.0nm

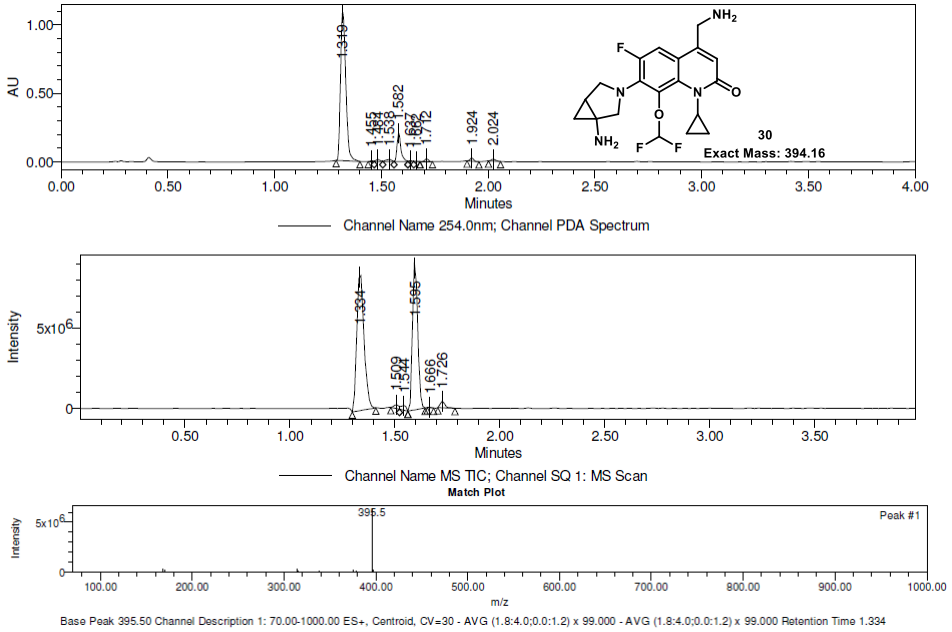
7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinoline-8-carbonitrile trifluoroacetic acid (28)



7-(1-Amino-5-methyl-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methoxyquinolin-2(1*H*)-one trifluoroacetic acid (29)



7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-8-(difluoromethoxy)-6-fluoroquinolin-2(1*H*)-one hydrochloride (30)

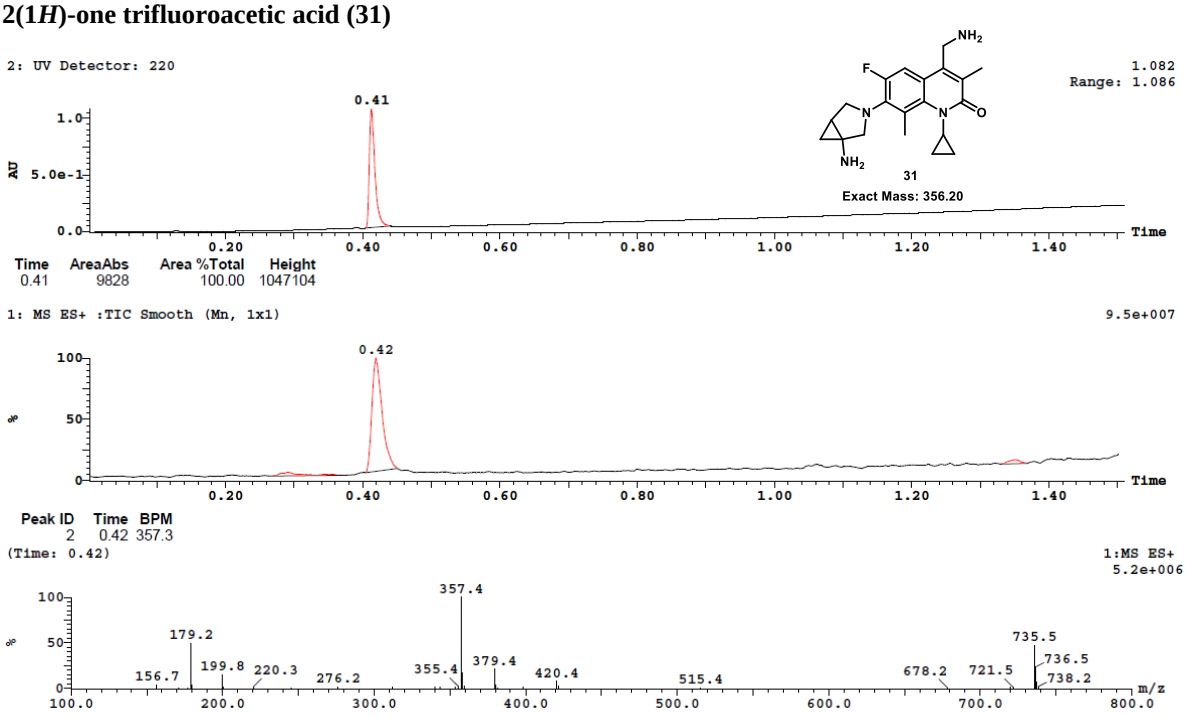


Base Peak 395.50 Channel Description 1: 70.00-1000.00 ES+, Centroid, CV=30 - AVG (1.8:4.0;0.0:1.2) x 99.000 - AVG (1.8:4.0;0.0:1.2) x 99.000 Retention Time 1.334

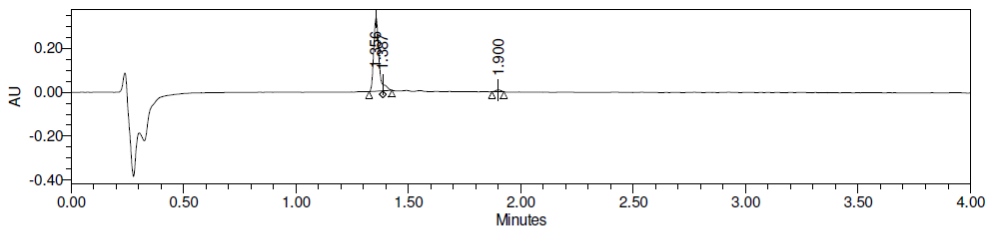
Peak Results
Channel: PDA Spectrum

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.319		1081844	1860873	82.49	PDA Spectrum	254.0nm
2	1.455		5778	6317	0.28	PDA Spectrum	254.0nm
3	1.484		15449	20345	0.90	PDA Spectrum	254.0nm
4	1.538		14691	28433	1.26	PDA Spectrum	254.0nm
5	1.582		203953	261930	11.61	PDA Spectrum	254.0nm
6	1.637		5844	7077	0.31	PDA Spectrum	254.0nm
7	1.662		3395	3584	0.16	PDA Spectrum	254.0nm
8	1.712		18788	22470	1.00	PDA Spectrum	254.0nm
9	1.924		22059	25773	1.14	PDA Spectrum	254.0nm
10	2.024		13156	19064	0.85	PDA Spectrum	254.0nm

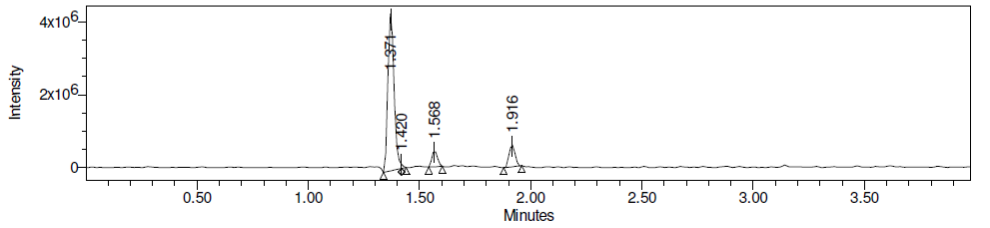
7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-3,8-dimethylquinolin-2(1*H*)-one trifluoroacetic acid (31)



7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-3-hydroxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (32)

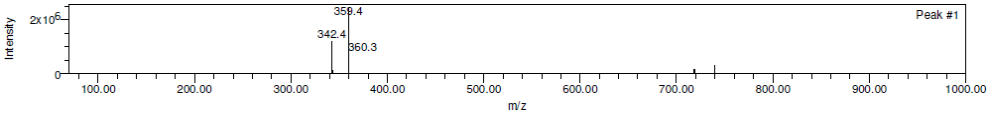


Channel Name 228.0nm; Channel PDA Spectrum

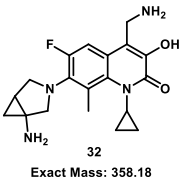


Channel Name MS TIC; Channel SQ 1: MS Scan

Match Plot



Base Peak 359.44 Channel Description 1: 70.00-1000.00 ES+, Centroid, CV=30 - AVG (2.0:4.0;0.0:1.2) x 99.000 - AVG (2.0:4.0;0.0:1.2) x 99.000 Retention Time 1.371



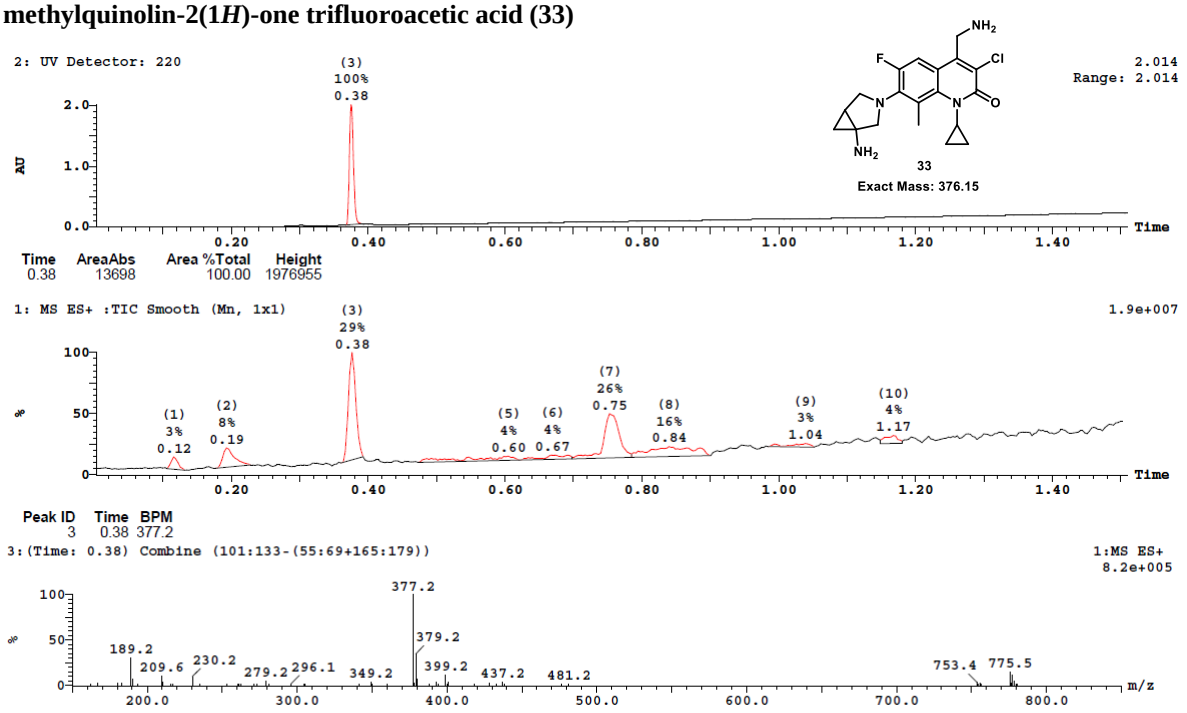
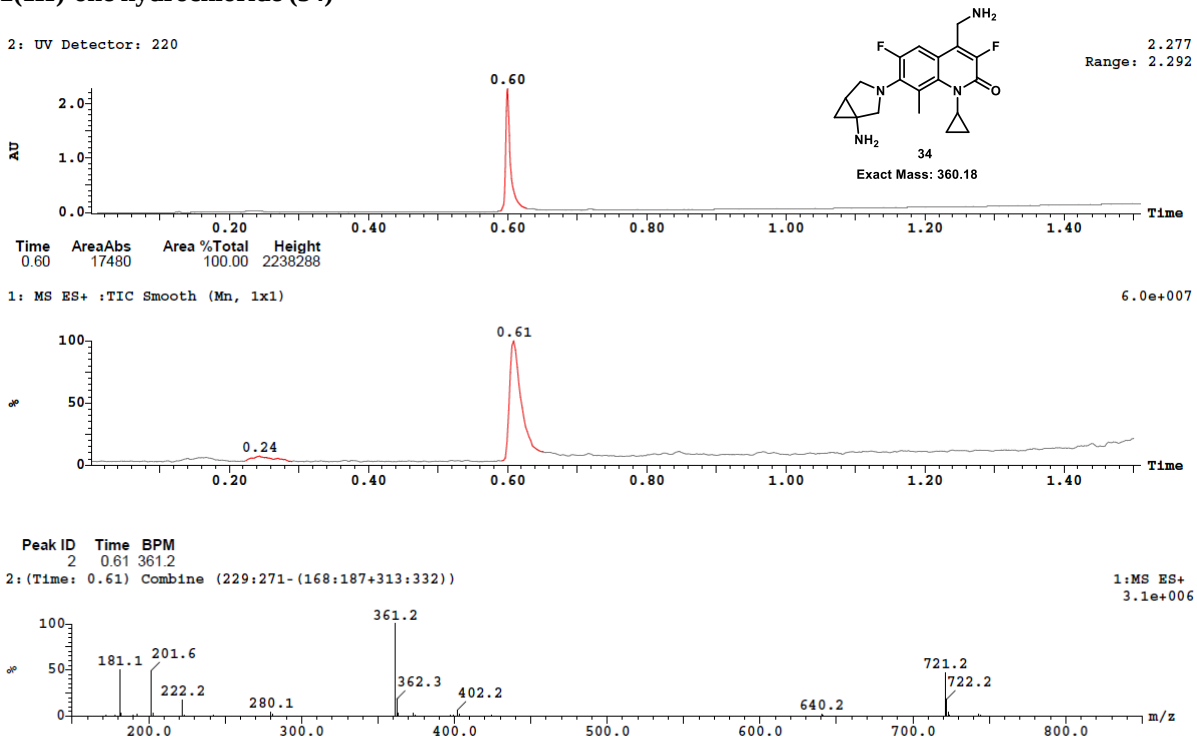
32

Exact Mass: 358.18

Peak Results
Channel: PDA Spectrum

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.356		335271	508717	91.39	PDA Spectrum	228.0nm
2	1.387		28324	34922	6.27	PDA Spectrum	228.0nm
3	1.900		8082	13004	2.34	PDA Spectrum	228.0nm

7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-3-chloro-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (33)



7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-3,6-difluoro-8-methylquinolin-2(1*H*)-one hydrochloride (34)