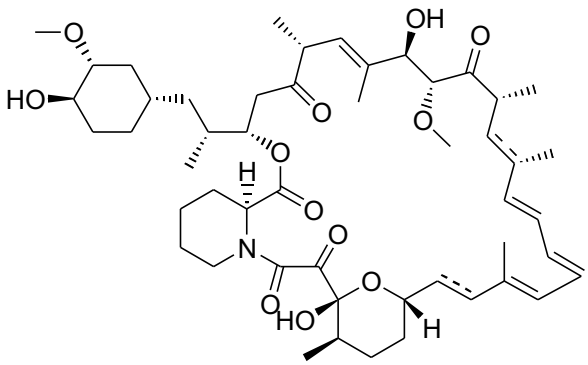
Certificate of Analysis



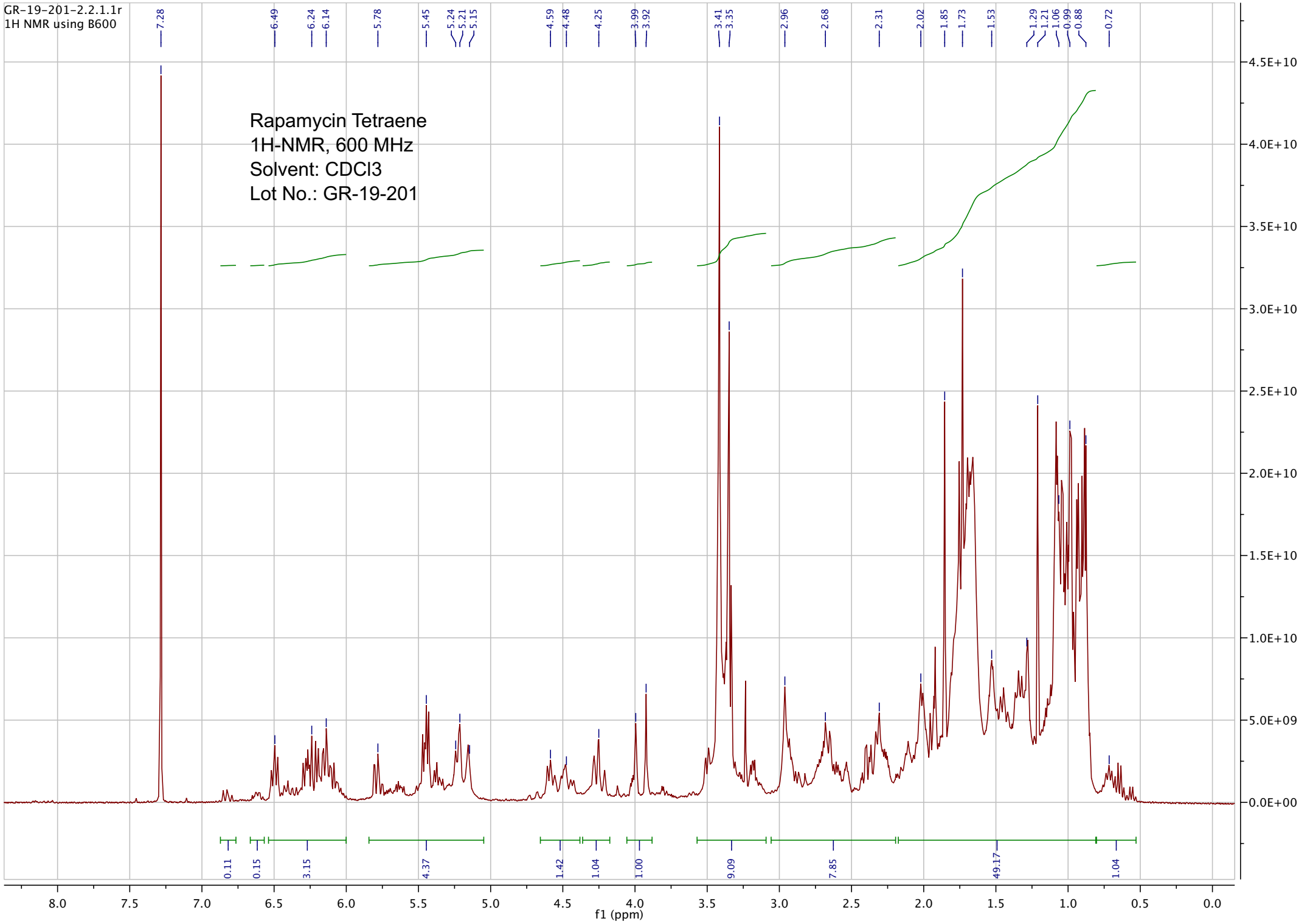
Name:	**Rapamycin Tetraene**
Lot#:	GR-19-201
Test Date:	12/17/2024 (re-test date: 12/17/2026)
CAS No.:	NA
MF:	$C_{50}H_{75}NO_{12}$
MW:	882.15
Appearance:	Off-White solid
Purity:	96.7% by HPLC (average of two sample preparations)
^{1}H-NMR:	Conforms (shows a mixture of position and Z-/E-isomers)
MS-ESI (-)	Conforms (shows peaks at m/z = 880.52 $[M-H]^-$, 940.54 $[M+CH_3COOH-H]^-$)
Storage	Store at -18°C in a dry place away from direct sunlight under nitrogen

Approved by:

Date: 01/07/2025

Viorica Rusu, QC/QA Manager

700, Collip Circle, The Research Park, London, ON, N6G 4X8
Ph: (647)4079078, e-mail: grusu@esschemco.com, www.esschemco.com


GR-19-201-2.2.1.1r
1H NMR using B600
Rapamycin Tetraene
1H-NMR, 600 MHz
Solvent: CDCl3
Lot No.: GR-19-201
7.28
6.49
6.24
6.14
5.78
5.45
5.24
5.21
5.15
4.59
4.48
4.25
3.99
3.92
3.41
3.35
2.96
2.68
2.31
2.02
1.85
1.73
1.53
1.29
1.21
1.06
0.99
0.88
0.72
0.11
0.15
3.15
4.37
1.42
1.04
1.00
9.09
7.85
49.17
1.04
f1 (ppm)

24.12.13.3106

MASS SPECTROMETRY

Sample Submission Form 2022-2023

The AIMS Mass Spectrometry Laboratory has no Biocontainment Level designation.
All samples of biological origin must therefore be inactivated and free from pathogens.

	Yes	No
Is sample of biological origin?	○	⊗
If yes, has sample been treated to inactivate pathogens?	○	○

Authorized Signature: [signature]

P.I. Name: Expert Synthesis Solutions

Account:

Name: Ghenadie Rusu

Email (Please type): grusu@esschemco.com

Sample ID: GR-19-201

Molecular Formula: $C_{50}H_{75}NO_{12}$ Molecular Mass: 882.15

Solubility: H_2O ☐ CH_3OH ☐ CH_3CN ☒ CH_2Cl_2 ☐ Other:

Structure / Sample information:

Rapamycin Tetraene

Please use the lowest ionization energy.

Thank you!

Ionization/Analysis:	EI	☐	ESI	☒	DART	☐	MALDI	☐	LC-MS ☐
Polarity:	+	☐	-	☒					GC-MS ☐
Accurate Mass:		☐							

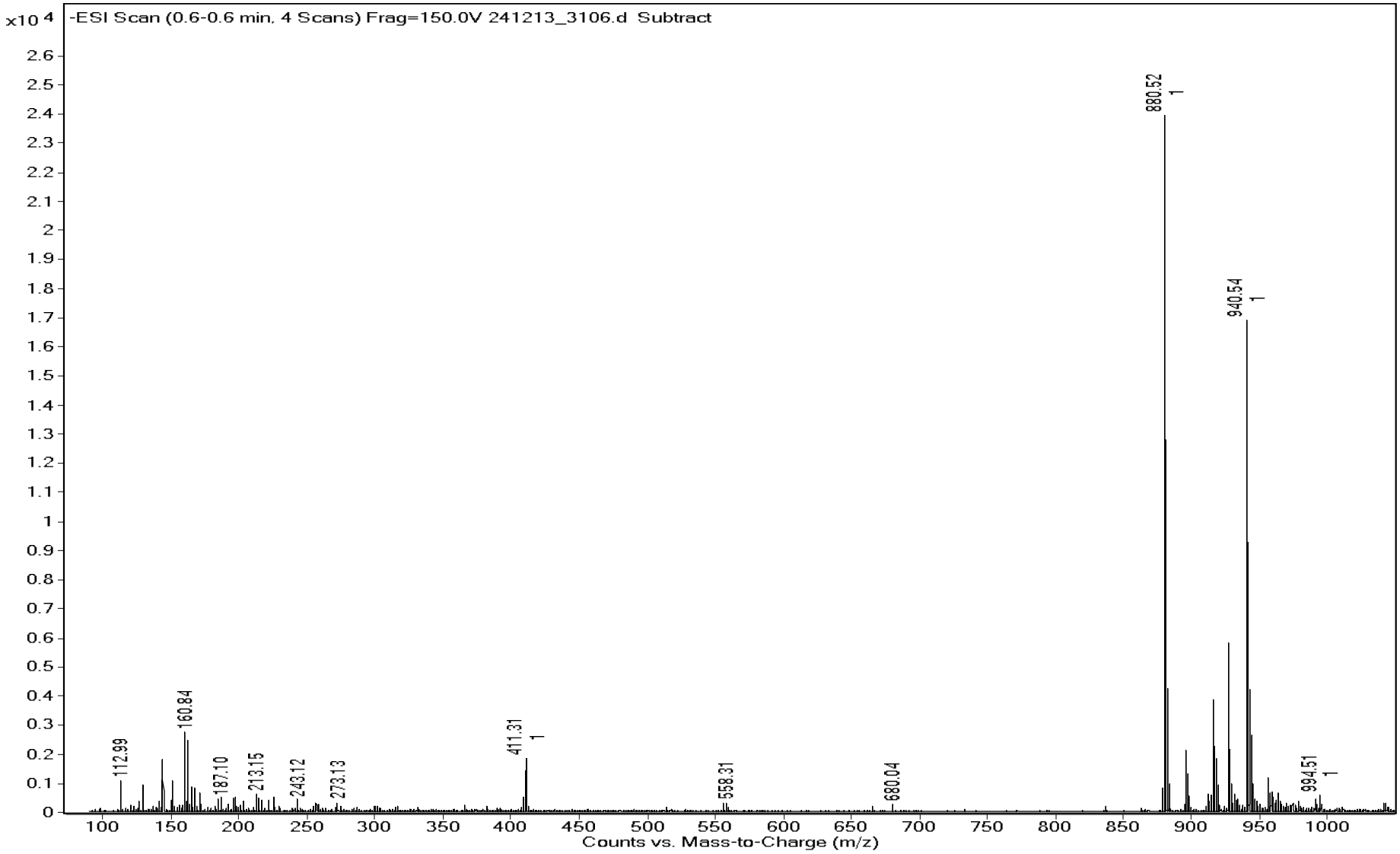
AIMS MASS SPECTROMETRY LABORATORIES

uoft.me/AIMS

CHEMISTRY UNIVERSITY OF TORONTO

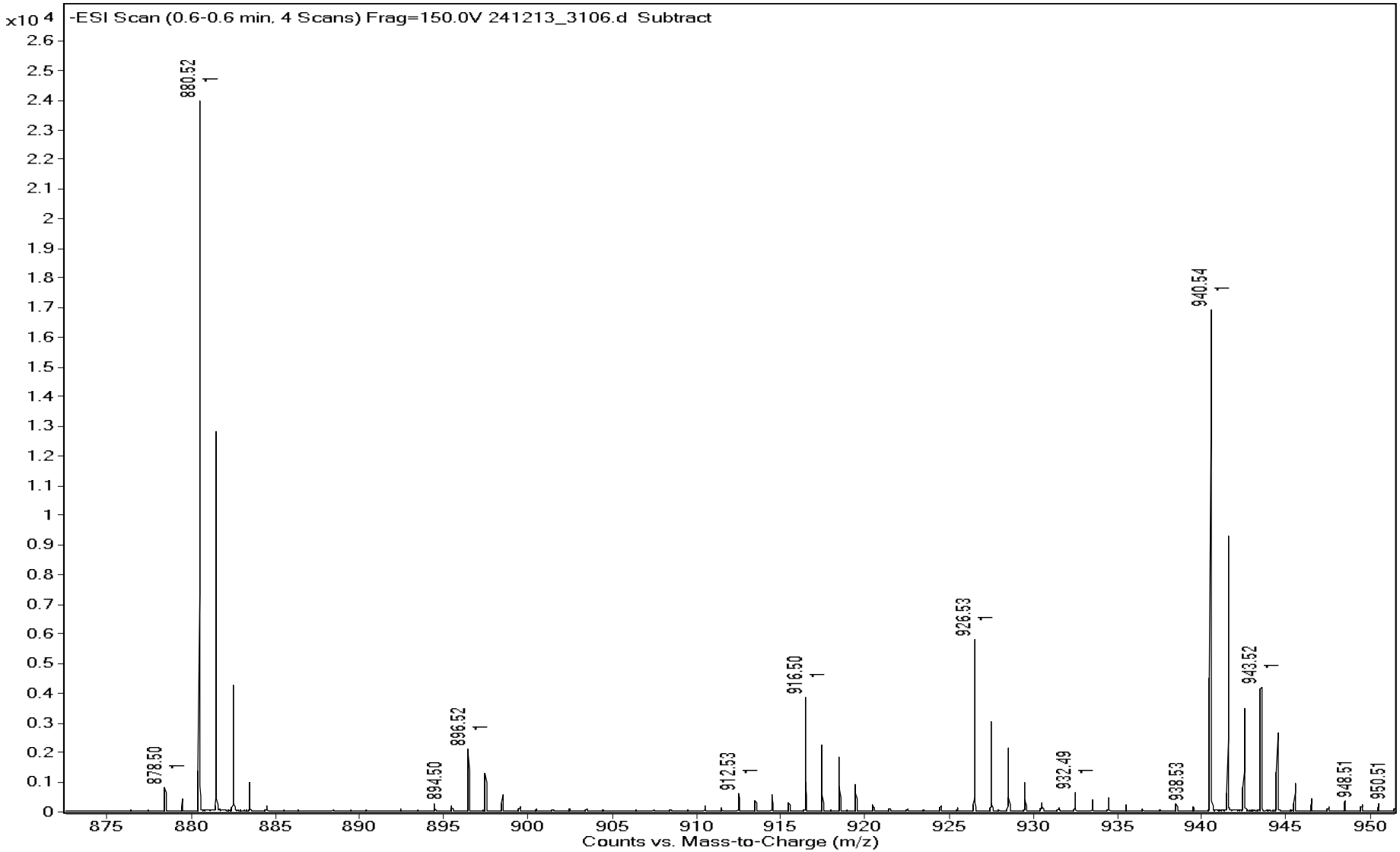
ESI Mass Spectrum

Sample Name	GR-19-201	**Data File**	241213_3106.d	**Acq Method**	HRMS_Negative.m
DA Method	AIMS_Accurate_Mass.m	**Instrument**	Agilent 6538 UHD	**Acq Date, Time**	13/12/2024 4:24:40 PM
Comment	ESI-				



ESI Mass Spectrum

Sample Name	GR-19-201	**Data File**	241213_3106.d	**Acq Method**	HRMS_Negative.m
DA Method	AIMS_Accurate_Mass.m	**Instrument**	Agilent 6538 UHD	**Acq Date, Time**	13/12/2024 4:24:40 PM
Comment	ESI-				



ESI-MS Report
Molecular Formula Generation (MFG)

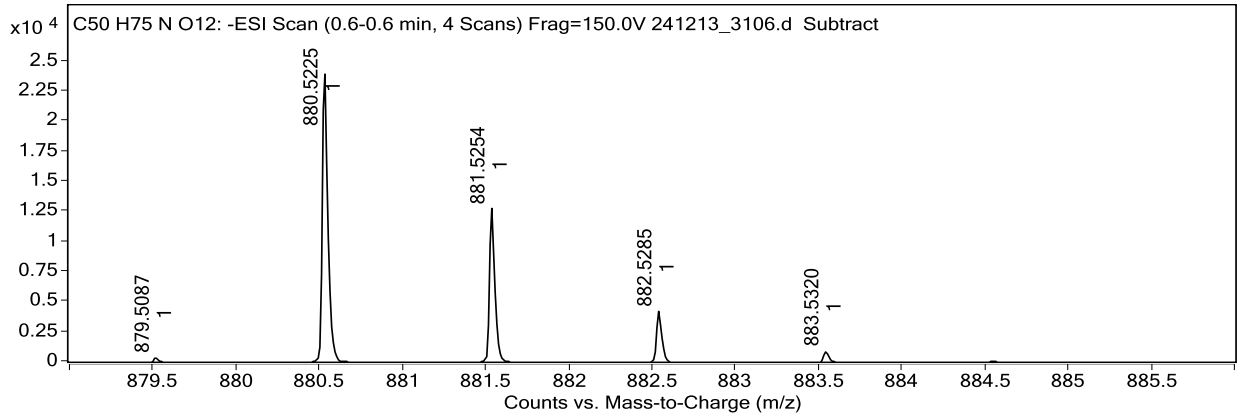
Sample Name	GR-19-201	**Data File**	241213_3106.d
Acq Method	HRMS_Negative.m	**DA Method**	AIMS_Accurate_Mass.m
Instrument	Agilent 6538 UHD	**Acq Date, Time**	13/12/2024 4:24:40 PM
Comment	ESI-		

Target Ion Species

Ion Species	*m/z*	*Ionic Formula*
(M-H)-	880.5225	C50 H74 N O12

MFG Calculator Results

Target m/z	*Ionic Formula*	*Calc m/z*	*+/- (mDa)*	*+/- (ppm)*	*DBE*	*MFG Score*
880.5225	C51 H70 N5 O8	880.5230	-0.5	-0.6	19.0	99.16
880.5225	C50 H74 N O12	880.5217	0.8	0.9	14.0	99.11
880.5225	C38 H78 N3 O19	880.5235	-1.0	-1.1	1.0	97.22
880.5225	C52 H66 N9 O4	880.5243	-1.8	-2.0	24.0	95.02
880.5225	C34 H74 N9 O17	880.5208	1.7	1.9	2.0	93.95
880.5225	C39 H74 N7 O15	880.5248	-2.3	-2.6	6.0	92.59
880.5225	C46 H70 N7 O10	880.5190	3.5	4.0	15.0	85.69
880.5225	C56 H70 N3 O6	880.5270	-4.5	-5.1	23.0	76.25
880.5225	C45 H74 N3 O14	880.5176	4.9	5.6	10.0	76.16



Predicted Isotope Match Table

Isotope	*m/z*	*Calc m/z*	Diff (mDa)	Abund (%)	Calc Abund (%)	+/-
1	880.5225	880.5217	0.8	100.0	100.0	0.0
2	881.5254	881.5250	0.4	52.8	55.8	3.0
3	882.5285	882.5280	0.5	17.6	17.7	0.1
4	883.5320	883.5309	1.1	4.0	4.1	0.1

Data File C:\CHEM32\1\DATA\VR011824\SIG12876.D
Sample Name: GR-19-201

```
==========================================================================
Acq. Operator   :
Acq. Instrument : Instrument 1                    Location : Vial 98
Injection Date  : 12/17/2024 1:49:13 PM
                                                  Inj Volume : 10.0 µl
Acq. Method     : C:\CHEM32\1\METHODS\VR121724_201.M
Last changed    : 12/17/2024 1:48:10 PM
Analysis Method : C:\CHEM32\1\METHODS\VR121724_201PM.M
Last changed    : 12/17/2024 3:36:25 PM by vrusu
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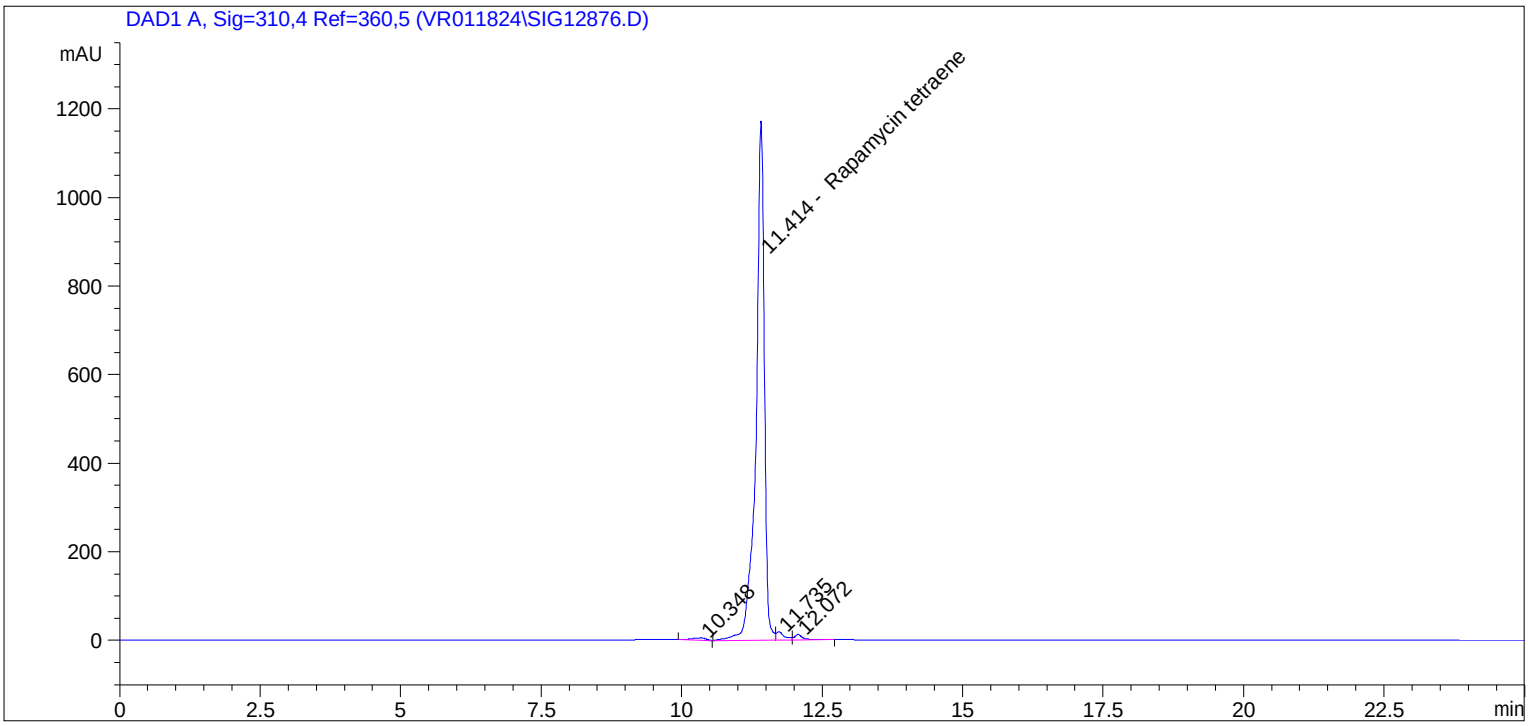


```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      12/17/2024 3:35:24 PM
Multiplier:                   :    1.603e3
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=310,4 Ref=360,5

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|----------------------
   1  10.348 BB      0.2327   86.57322   0.6912 ?
   2  11.414 BV      0.1506 1.21133e4   96.7145 Rapamycin tetraene
   3  11.735 VV      0.1461  193.37875   1.5440 ?
   4  12.072 VB      0.1415  131.55577   1.0504 ?

Totals :                   1.25248e4


==========================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\VR011824\SIG12877.D
Sample Name: GR-19-201 spl-2

```
==========================================================================
Acq. Operator   :
Acq. Instrument : Instrument 1                    Location : Vial 99
Injection Date  : 12/17/2024 2:21:14 PM
                                                  Inj Volume : 10.0 µl
Acq. Method     : C:\CHEM32\1\METHODS\VR121724_201.M
Last changed    : 12/17/2024 2:16:32 PM
Analysis Method : C:\CHEM32\1\METHODS\VR121724_201PM.M
Last changed    : 12/17/2024 3:36:25 PM by vrusu
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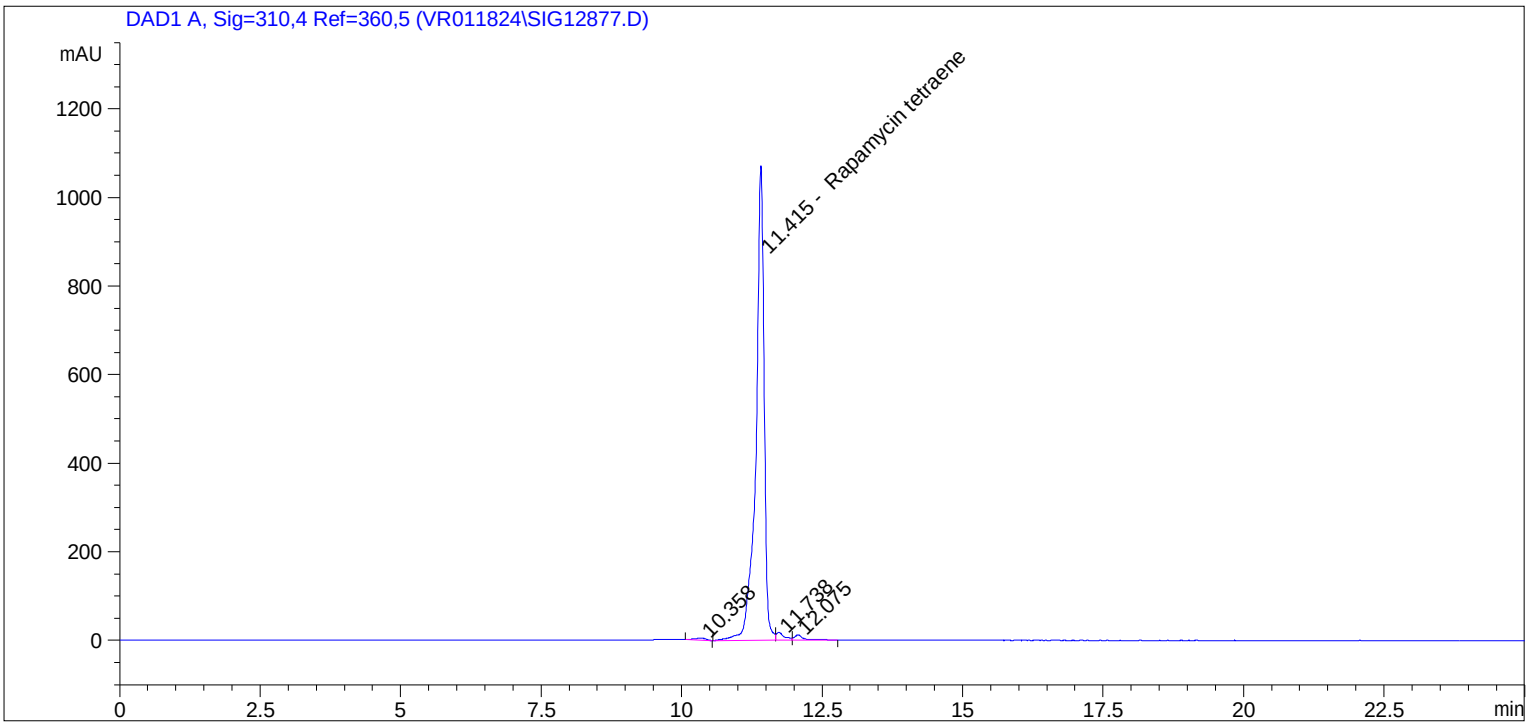


```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      12/17/2024 3:35:24 PM
Multiplier:                   :    1.603e3
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=310,4 Ref=360,5

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|----------------------
   1  10.358 BB      0.1949   65.90385   0.5802 ?
   2  11.415 BV      0.1495 1.09839e4   96.7036 Rapamycin tetraene
   3  11.738 VV      0.1443  180.04883   1.5852 ?
   4  12.075 VB      0.1442  128.45981   1.1310 ?

Totals :                   1.13583e4


==========================================================================
                          *** End of Report ***
```